

**PHYSICAL PROPERTIES OF STARCH
IN CONCENTRATED SYSTEMS SUCH
AS DOUGH AND BREAD**

Ann-Charlotte Eliasson

LUND 1983

PHYSICAL PROPERTIES OF STARCH IN CONCENTRATED
SYSTEMS SUCH AS DOUGH AND BREAD

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DOKUMENTTITEL OCH UNDERTITEL Physical properties of starch in concentrated systems such as dough and bread			
SAMMANFATTNING The aim of this work has been to contribute to the understanding of the role of starch in bread-making and in staling of bread. Gelatinization and retrogradation of starch in concentrated aqueous systems have therefore been studied using differential scanning calorimetry (DSC) and stress-relaxation technique. An aqueous suspension of wheat starch exhibits three endothermic transitions. The high-temperature endotherm is due to an amylose-lipid complex whereas the other two endotherms are directly related to the gelatinization process. According to the thermal behaviour, it is natural to describe the gelatinization as a diluent-dependant melting of the crystalline regions in the starch granules. The low temperature endotherm emerges from a cooperative melting in excess water, and the second endotherm represents a kind of melting phenomenon when no excess water is present. The rheological properties of concentrated wheat starch gels have been measured in stress-relaxation experiments, and on the basis of the results, the starch gel is described as a composite material with more or less swollen granules dispersed in an aqueous amylose solution.			
NYCKELORD Gelatinization, retrogradation, wheat starch, differential scanning calorimetry, amylose-lipid complex			
DOKUMENTTITEL OCH UNDERTITEL – SVENSK ÖVERSÄTTNING AV UTLÄNDSK ORIGINALTITEL Stärkelsens fysikaliska egenskaper i koncentrerade system sådana som deg och bröd			
TILLÄMPNINGSSOMRÅDE Resultaten rörande stärkelsens gelatinisering och retrogradering i koncentrerade system och inverkan av gluten och lipider på dessa processer kan vara av intresse för alla som använder stärkelse, t ex inom livsmedelsindustrin (speciellt bageri)			
NYCKELORD Gelatinisering, retrogradering, lipider, gluten, livsmedel, bageri			
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Interaction between starch and gluten was also studied. Gluten itself is only marginally influenced by heat, i.e. very small transition enthalpies are observed by DSC. The presence of gluten was found to increase the gelatinization temperature and decrease the gelatinization enthalpy of wheat starch.

In studies of the interaction between starch and lipids it was found that the formation of amylose-lipid complexes results in a delay in the gelatinization process, as was obvious from decreased swelling of the starch granules, increased "pasting" temperature, reduction of viscosity, and decreased gelatinization enthalpy at low water contents. Differences between wheat starch varieties in this respect were observed.

The retrogradation of starch is reduced by the presence of gluten as well as lipids. Depending on the structure of the lipid, amylose-lipid complexes with different thermal properties and water-holding capacities can be formed, and these differences are proposed to be relevant for their anti-staling effect.

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SYSTEMS SUCH AS DOUGH AND BREAD

BY

ANN-CHARLOTTE ELIASSON



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PUBLICATIONS

This thesis is based on the following papers, which will be referred to by Roman numerals in the text:

- I Effect of Water Content on the Gelatinization of Wheat Starch.
A.-C. Eliasson
Stärke 32 (1980) 270-272.
- II Thermal Stability of Wheat Gluten.
A.-C. Eliasson and P.-O. Hegg
Cereal Chem. 57 (1980) 436-437.
- III Some Effects of Starch Lipids on the Thermal and Rheological
Properties of Wheat Starch.
A.-C. Eliasson, T.L.-G. Carlson, K. Larsson and Y. Mieziš
Stärke 33 (1981) 130-134.
- IV On the Possibility of Modifying the Gelatinization Properties of
Starch by Lipid Surface Coating.
A.-C. Eliasson, K. Larsson and Y. Mieziš
Stärke 33 (1981) 231-235.
- V Rheological Properties of Concentrated Wheat Starch Gels.
A.-C. Eliasson and L. Bohlin
Stärke 34 (1982) 267-271.
- VI Differential Scanning Calorimetry Studies on the System Gluten-Wheat
Starch. I. Effect of Gluten on the Gelatinization of Wheat Starch.
A.-C. Eliasson
J. Cereal Sci., accepted for publication.
- VII Differential Scanning Calorimetry Studies on the System Gluten-Wheat
Starch. II. Effect of Gluten and Surfactants on the Crystallization
of Wheat Starch during Ageing of the Starch Gel.
A.-C. Eliasson
J. Cereal Sci., accepted for publication.
- VIII Gelatinization Properties of Different Size Classes of Wheat Starch
Granules Measured with Differential Scanning Calorimetry.
A.-C. Eliasson and R. Karlsson
Stärke, in press.

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1. INTRODUCTION

Starch makes up almost 70% of wheat flour, and although it is the major component in dough and bread, the understanding of the role starch plays in the baking process is still far from complete. The behaviour of starch in diluted aqueous systems has been thoroughly studied, whereas concentrated systems, which would constitute a better model for dough and bread, have been neglected.

When starch is heated in the presence of water, it undergoes characteristic changes resulting in the formation of a gel, the well-known *gelatinization* process. Furthermore, a starch gel exhibits changes with time involving crystallization of the starch components, so-called *retrogradation*. These processes are of fundamental technical importance in several applications. The present work has been focused on the gelatinization involved in the bread making process and the retrogradation related to the staling of bread. As a basis for these applications, it was considered of value to start with thermal and rheological characterization of concentrated starch-water systems, in order to obtain a better understanding of gelatinization and retrogradation in well-defined systems.

Differential scanning calorimetry (DSC) combined with a stress relaxation technique developed at our department were used to study the gelatinization as well as the retrogradation. Other thermal transitions, in addition to the gelatinization transition, have recently been discovered, and the investigation of these additional transitions has also constituted an important part of this thesis. Soon after the present work was started, a new molecular description of the gelatinization process was published, which has been most useful in rationalizing the experimental findings.

In order to obtain a better understanding of the role starch plays in bread-making and in the staling of bread, it was natural to introduce other components into the starch-water system. The interaction between starch and lipids, as well as between starch and wheat gluten was therefore also studied.

2. THE STARCH GRANULE

Higher plants form starch in special cells, amyloplasts, in the form of water-insoluble particles, starch granules. The size and morphology of these granules are plant specific. In wheat starch, the granules are of two types: small spherical granules with a diameter less than $8\text{--}10\text{ }\mu\text{m}$ (B-granules), and large lenticular granules with maximum diameter around $20\text{ }\mu\text{m}$ (A-granules). A demonstration of the bimodal size distribution of starch granules in wheat is given in Fig. 1.

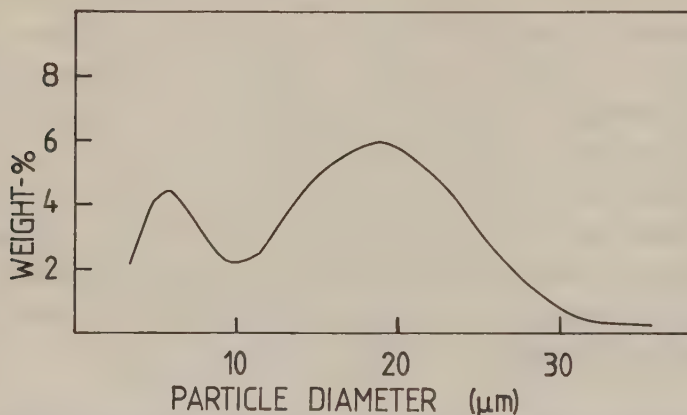


Fig. 1. The size distribution of starch granules from the wheat variety *Amy* (VIII).

The starch granules are built up from mainly two types of D-glucose polymers, amylose and amylopectin. Amylose is an essentially [1] linear polymer of glucose containing α - 1:4 - glucosidic linkages, whereas amylopectin is a highly branched polymer containing both α - 1:4 - and α - 1:6 - glucosidic linkages.

Starch is a semi-crystalline material, although the structure of the starch granule on the molecular level is still unknown. The crystalline nature of the starch granule structure can be observed by X-ray diffraction. Depending on the plant origin of the starch, A-, B- or C-patterns are obtained. The A-pattern is exhibited by starches of cereal origin, the B-pattern by tuber starch (e.g. potato) and retrograded starch, and the C-pattern, which is an intermediate form between the A- and B-types, is characteristic of e.g. bean starches. The structure of the native starch has been claimed to be a six-

residue double helix built up of amylopectin branches [1-4]. However, of the components only amylose has been crystallized to give the X-ray patterns [5]. The differences between the A- and B-patterns are due to the lateral packing of the helices and the water content. The A-amylose crystallizes in an orthorhombic unit cell with a slightly distorted hexagonal packing, and with 8 water molecules per unit cell. The B-amylose crystallizes in a hexagonal unit cell, with a much more open hexagonal packing and 36 water molecules per unit cell [5]. The double helix proposed by Sarko and Wu [5] for the B-pattern is illustrated in Fig. 2.

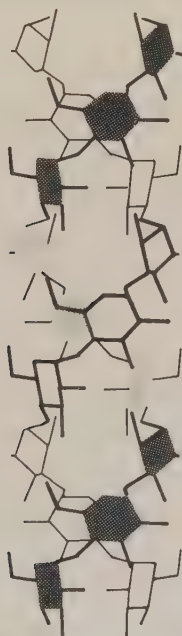


Fig. 2. The structure of amylose in B-starch as proposed by Sarko and Wu [5].

Due to the crystalline character, the starch granules show birefringence in polarized light as shown in Fig. 3.

Starch granules of cereal origin contain small amounts of non-carbohydrate material. Wheat starch granules have thus been reported to contain about 1% (w/w) of lipids in the form of an amylose-lipid inclusion complex [6,7]. This complex will be discussed in detail in section 3.3. Lipids and proteins, which might be remnants of the biomembrane on the starch granule in the

amyloplast, are also present on the surface of the wheat starch granule.

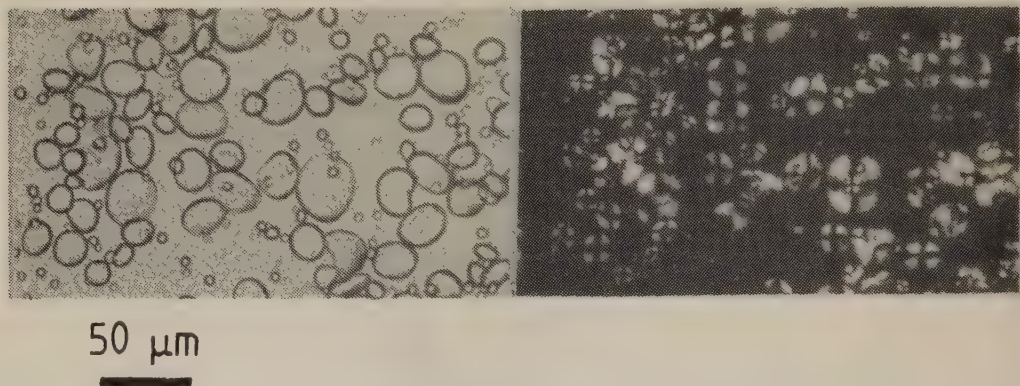


Fig. 3. Wheat starch viewed in the microscope using normal (left) and polarized (right) light.

The lipids on the surface of wheat starch granules were separated (III) and it was found that they form bilayers in water, a characteristic feature of membrane lipids. The amount of lipids obtained from the granule surface ($< 0.1\%$ (w/w)) was somewhat lower than the calculated amount which is necessary for forming a coherent lipid bilayer. The protein content was found to be less than 0.5% (w/w) and some of the proteins present on the starch granule surface were identified by electrophoresis as gliadins. The results in (III) also show that this adhering material differs between wheat starch varieties.

The presence of material adhering to the wheat starch granules has been demonstrated by scanning electron microscopy (SEM) [8]. Measurements of the zeta potential [9] have shown variations with pH, with an isoelectric point at pH 3.7 for B-granules.

3. GELATINIZATION

When native starch granules are suspended in water at room temperature, a small amount of water is reversibly absorbed, which results in a minor swelling (the lenticular granules of wheat, for example, have been reported to increase in diameter by approximately 30% [1]). When the temperature is then increased to the well-known gelatinization temperature, there is a drastic increase in the swelling, which is no longer reversible. Using the

polarizing microscope, it is seen that the granules lose their birefringence over a temperature range of about 5-15 degrees. The X-ray diffraction pattern is also lost during the gelatinization. In the presence of certain salts, e.g. 2.5 - 3.0 M calcium chloride [10], starch may gelatinize at room temperature.

During gelatinization amylose molecules leave the starch granule, and there is a correlation between "solubility" and "swelling power" (see e.g. ref. [11]). In a study of cold gelatinization, Lindqvist [12] even found that the amylose leakage is a necessary condition for gelatinization to occur. If the leakage of amylose is blocked, the gelatinization process is inhibited. This has also been observed in the case of thermal gelatinization [13].

Light microscopy and SEM were recently used by Bowler et al. [14] to study the morphological changes occurring when lenticular wheat starch granules are heated, and they found that the swelling is a two-stage process. First, there is a radial expansion, which results in flattened discs. Then follows a tangential expansion, which gives complex puckered granules. Individual starch granules were still observed after heating to 97°C. These morphological changes, which must be important for the rheological properties, are illustrated in Fig. 4.

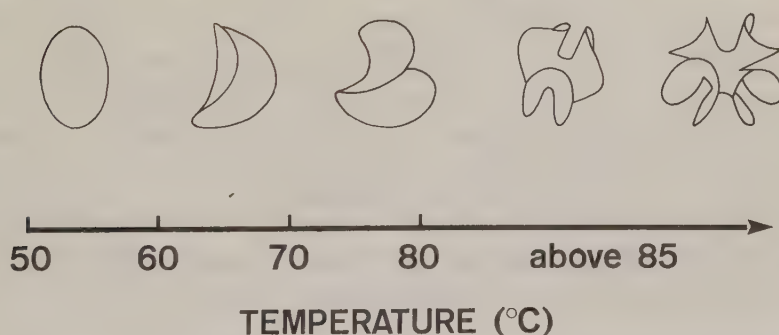


Fig. 4. The morphological changes occurring when large wheat starch granules are heated in excess water (from Bowler et al. [14]).

The viscosity of the starch suspension increases during the gelatinization towards gel formation. The structure of the gel changes with time due to crystallization of starch molecules, a phenomenon known as retrogradation.

Examples of methods, which have been used for studying starch gelatinization, are birefringence end point temperature (BEPT) [15], proton magnetic resonance [16], light scattering [17], viscosity methods such as the Brabender Amylograph technique [15] and thermal methods (differential thermal analysis (DTA) and DSC). One limiting factor with many of these methods (e.g. BEPT and viscosity measurements) is that they can only be used successfully in diluted starch suspensions (often less than 10% (w/w)). In the thermal methods (DTA and DSC), however, both the water:starch ratio and the temperature interval investigated can be varied within wide ranges.

3.1 Gelatinization as Observed by Differential Scanning Calorimetry

The use of DSC for studying starch gelatinization was first reported by Stevens and Elton [18], who observed an endothermic transition, which was interpreted as resulting from a melting process [1].

The present work was started by a DSC-study on gelatinization of wheat starch at different water:starch ratios (I). During the course of this work, Donovan [19] published a similar study on potato starch. Subsequently, numerous papers were published on the use of DSC for studying gelatinization of starch in the presence of limited amounts of water [20-22].

At intermediate water:starch ratios ($\sim 2.0 - 0.5$ g water/g starch), three endotherms are observed for wheat starch (I; Fig. 5), whereas only two endothermic transitions are observed for potato starch [19]. The high-temperature endotherm (transition III in Fig. 5) is due to a transition of the amylose-lipid complex [23], which is present in wheat starch, but not in potato starch. When lipids capable of complexing with amylose are added to potato starch, the high-temperature endotherm is observed for this starch as well (IV). The discussion in this section will be focused on the two endotherms at the lower temperatures (transitions I and II in Fig. 5), and the amylose-lipid complex will be discussed in section 3.3.

Thermal behaviour has been found to be similar for all starch types investigated: wheat (I), potato [19], adzuki bean, smooth pea and lentil [21], and rice [24]. At high water: starch ratios an endotherm at the gelatinization temperature (T_I , for the notations see Fig. 5) is observed. The enthalpy of this endotherm (ΔH_I) is, within certain limits, independent of the water content. When the water content is decreased below a certain level, a

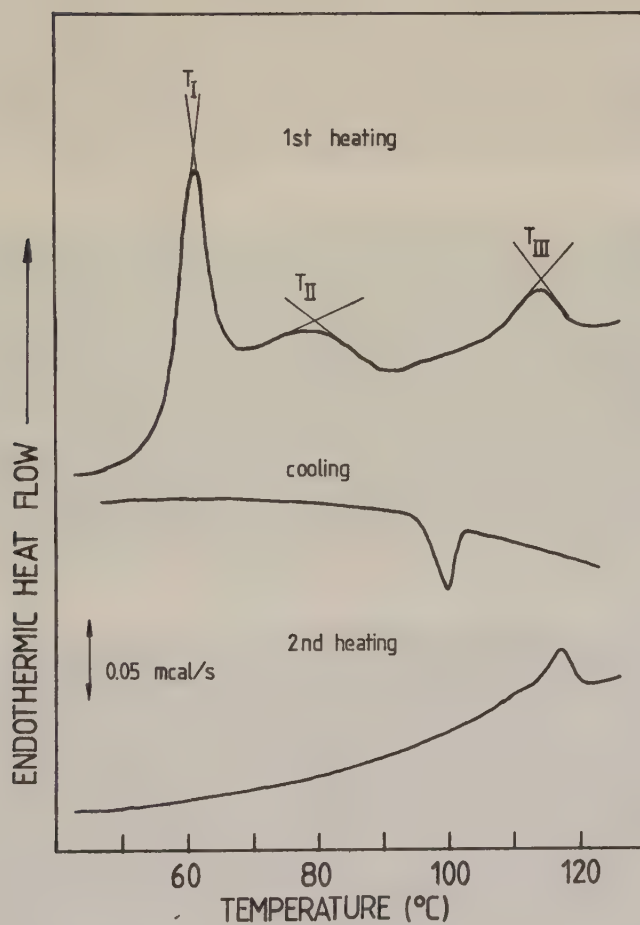
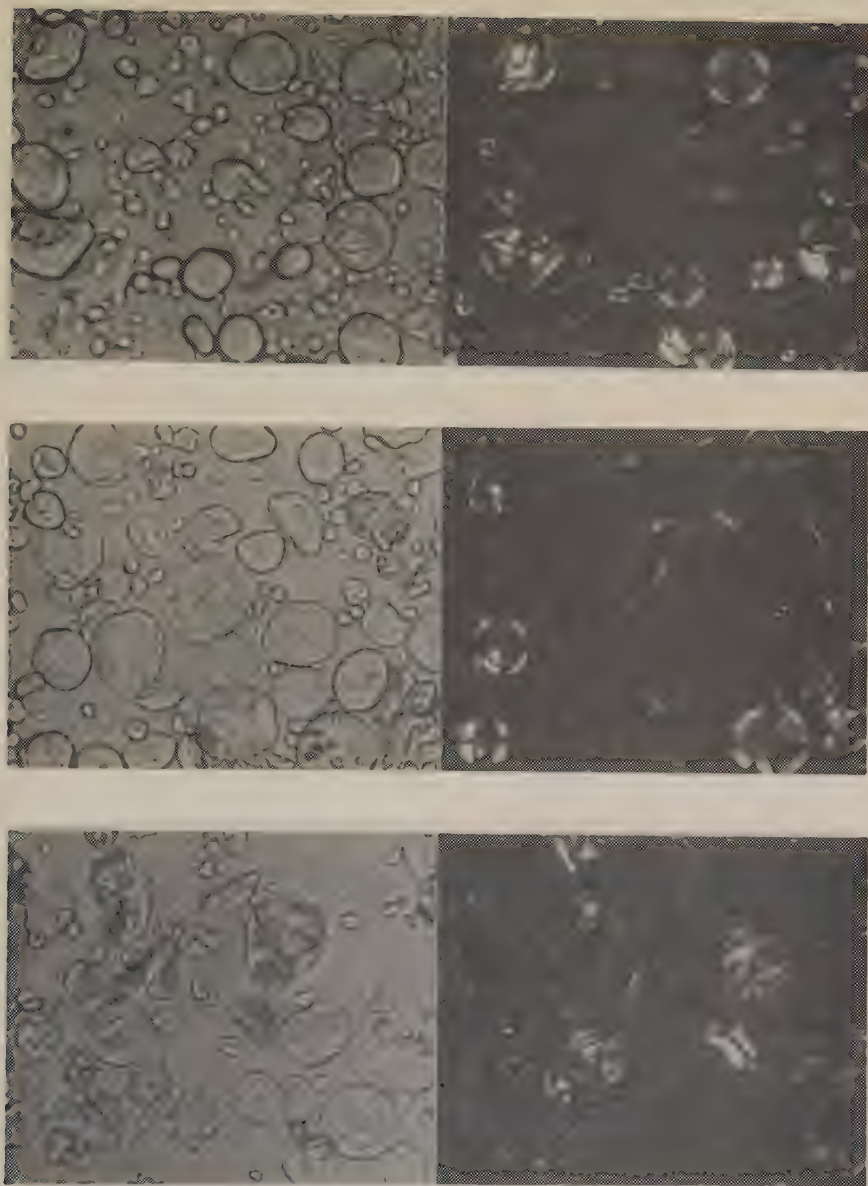


Fig. 5. DSC-thermograms of heating, cooling and reheating wheat starch. The water:starch ratio was 1:1, and the scanning rate was 10°C/min.

shoulder develops on the gelatinization peak. The gelatinization enthalpy decreases linearly with the water content, until, at a critical water content, no gelatinization endotherm is observed at all. The enthalpy of the second endotherm (ΔH_{II}) shows a maximum at a certain water content. Donovan [19] calculated the enthalpies as a function of water molecules per hexose unit, and found that four water molecules per hexose unit are necessary for gelatinization to occur at all, whereas 14 water molecules per hexose unit are necessary for complete gelatinization. The minimum water content for gelatinization and the water content necessary for complete gelatinization observed for wheat starch correspond to 4 and 20 water molecules per hexose unit, respectively (I). The maximum in ΔH_{II} occurs at 4 water molecules per hexose unit for potato starch [19], and at 7 water molecules per hexose unit for wheat starch (I).

The gelatinization temperature (T_I) seems to be independent of the water content, whereas the temperature of the second transition (T_{II}) increases regularly with decreasing water content. This variation in T_{II} has led several investigators [10,19,21,25] to compare the gelatinization of starch with the melting of a crystalline polymer in the presence of a diluent according to the theory of Flory [26]. The qualitative aspects of this approach will be discussed further below, and this description is in close agreement with the discussion of Evans and Haisman [10] for potato starch. The starch granules are considered as semi-crystalline particles, in which crystalline regions (crystallites) alternate with amorphous regions. The degree of crystallinity of native potato starch, estimated from X-ray diffraction data, was found to be 24% [27] whereas the corresponding value of wheat starch was somewhat higher. The degree of order within each crystalline region can vary, and thus the melting point at a given water content should be expected to differ both between crystallites in the same granule and between crystallites in different granules. During heating of a starch sample, the melting point of the least stable crystallite is reached first, and it thus starts to melt. The order-disorder transition causes diffusion freedom of the molecules, and when amylose leaves the granule, it absorbs water. The water content of the granule thus increases successively, and therefore the melting point of the remaining crystallites decreases. Due to this cooperative effect, a single granule gelatinizes during a narrow temperature interval, whereas the gelatinization temperature range in a population of starch granules is broader.

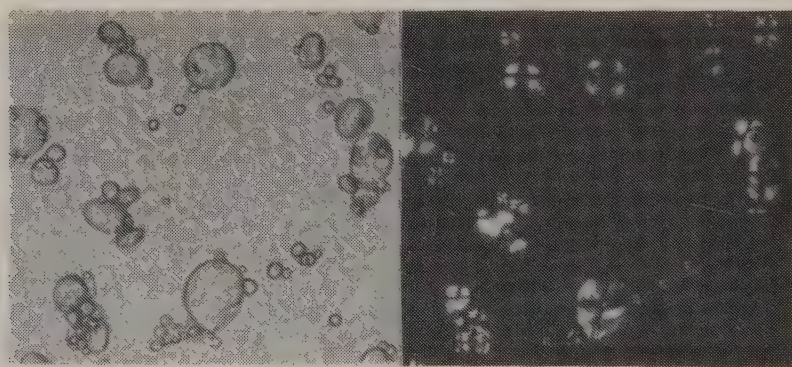


50 μm

Fig. 6. The appearance of wheat starch granules after heating at a water: starch ratio of 1:1 to (from top to bottom) 68.5°C, 75°C and 90°C. The gels were suspended in excess water before the photographs were taken.

This description is only valid when there is enough free water present, i.e. when only one endotherm is observed on the DSC-thermogram. When the water content is lower, the gelatinization proceeds in a somewhat different way. In the beginning, when there is enough water, the gelatinization occurs as described above. Later on, a point is reached, where there is no more water available to be absorbed. The remaining crystallites thus melt according to their melting point at the actual water content. There are thus two endotherms on the DSC-thermogram, one at low temperature (T_I) where the melting of crystallites occurs in excess water, and one at increasing temperatures (T_{II}), due to the melting of crystallites in the presence of limited amounts of water.

The existence of ordered regions at high temperatures when limited amounts of water are present is illustrated in Figs. 6 and 7, using the polarizing microscope. At a water:starch ratio of 1:1 (Fig. 6), birefringent regions still exist at a temperature of 90°C. At a water:starch ratio of 0.4:1, the starch granules are birefringent even after heating to 132°C (Fig. 7). Moreover the corresponding thermogram showed that no gelatinization endotherm was present at this low water content (I).



50 μm

Fig. 7. Wheat starch heated to 132°C at a water:starch ratio of 0.4:1.

The gelatinization enthalpy (ΔH_I) and temperature (T_I) for some wheat starch preparations are given in Table 1. The data illustrate large differences between wheat starch samples. The gelatinization temperature ranges from 57.0 to 69°C, and the gelatinization enthalpy from 1.93 to 5.2 cal/g.

Some of the differences could be explained by variations in scanning rate (e.g. the high gelatinization temperature obtained at a scanning rate of 20°C/min). The enthalpies are only marginally affected by the scanning rate in the range given in Table 1 [19],(I). It was also found that any influence of the scanning rate is the same for the two transitions discussed above, since the ratio between these enthalpies is independent of the scanning rate in the range of 5 to 20°C/min (I).

TABLE 1
Gelatinization properties of different wheat
starch preparations measured by DSC

Wheat starch	Scanning rate (°C/min)	Water:starch ratio	T_I (°C)	ΔH_I (cal/g)	Ref.
Wheat	20	2:1	69	2.4	[18]
Commercial	10	4:1	59.0	2.4	[22]
Commercial	8	2:1	67	5.2	[20]
From milled flour	5	4:1	57.0	2.26 ± 0.15	[28]
From whole grain	5	4:1	57.8	1.93 ± 0.14	[28]
Amy					(VIII)
Whole sample	10	3:1	61.6	3.03 ± 0.10	
Large granules	10	3:1	61.2	3.35 ± 0.04	
Small granules	10	3:1	62.4	3.15 ± 0.03	

Even though the influence of experimental conditions during the recording of the DSC-thermogram at T_I and ΔH_I is accounted for, differences still exist between various types of wheat starches. The differences may be due to several factors such as amount of damaged starch [18], preparation procedure (e.g. influence of steeping temperature [29]) and wheat variety. The influence of the wheat variety in turn may depend on lipid content, surface coating and granule size distribution. The significance of lipid interaction will be discussed in section 3.3. The influence of the granule size distribution on the gelatinization properties was examined in (VIII), and it was found that at a high water:starch ratio (3:1), ΔH_I was somewhat higher for the large granules, whereas at a lower water:starch ratio (1:1), there was no difference in enthalpies between small and large granules (VIII). The gelatinization temperature, however, was sensitive to the granule size

distribution. The small granules started to gelatinize at a lower temperature than the large granules. The peak temperature was also higher, and the whole gelatinization interval was thus broader for the small granules. This was most evident at high water content, where the gelatinization temperature interval was 14°C for the small granules and 9°C for the large ones. The appearance of the DSC-thermograms for small and large granules differed strikingly (Fig. 8). The large granules gave rise to a high and narrow gelatinization peak, whereas the small granules gave rise to a low and broad gelatinization peak. The DSC-thermogram for the original wheat starch sample was somewhere in between. The smaller gelatinization interval and the narrower DSC-peak for the large granules indicate that these granules are more homogeneous compared to the small granules. Results reported earlier concerning gelatinization temperatures of small and large wheat starch granules are somewhat different from those reported here (VIII). When gelatinization temperatures are measured microscopically, the whole gelatinization interval of the small granules is found to be displaced towards higher temperatures [1,30]. The higher peak and conclusion temperatures of small granules are confirmed by the DSC-measurements in (VIII). The discrepancy regarding the gelatinization onset temperature may be due to differences in the sensitivities of the methods. The start of birefringence reduction could be difficult to detect in the case of small granules. When thermal properties of different wheat starches are compared, it thus seems necessary to take the granule size distribution into consideration.

3.2 Rheological Changes during Gelatinization

The increase in viscosity of a starch suspension during heating, the changes during a holding period at about 95°C and the consistency during the subsequent cooling are often used to characterize starch gels. An example of such a viscosity curve for wheat starch is given in Fig. 9.

The rheological behaviour of starch gels having low concentration (i.e. 10% starch or less) studied under well-defined flow regimes has recently been reported [31-34]. The gelatinized starch-suspensions are shear-thinning fluids and at low shear rates the flow curves (shear stress *versus* shear rate) also exhibit a yield stress. Deviations from Newtonian behaviour were observed above a certain starch concentration, which was found to be specific for each starch. In the case of wheat flour this concentration was 3.0%, which is in good agreement with the estimated concentration necessary for

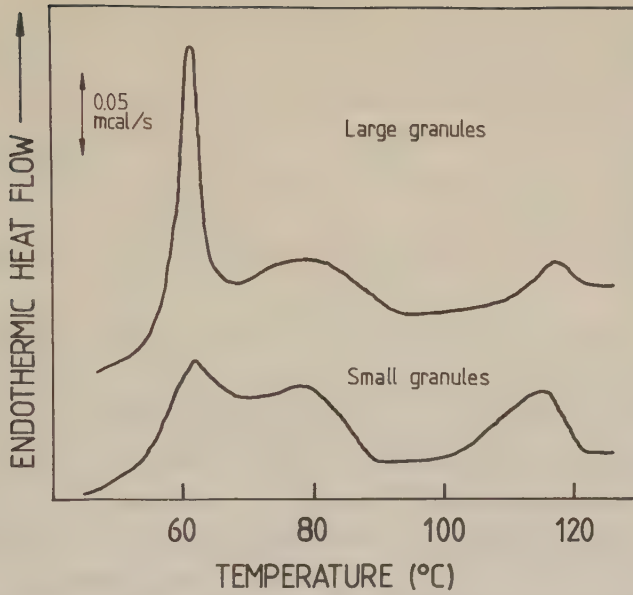


Fig. 8. DSC-thermograms of small and large wheat starch granules from *Amy*. The water:starch ratio was 1:1 (VIII).

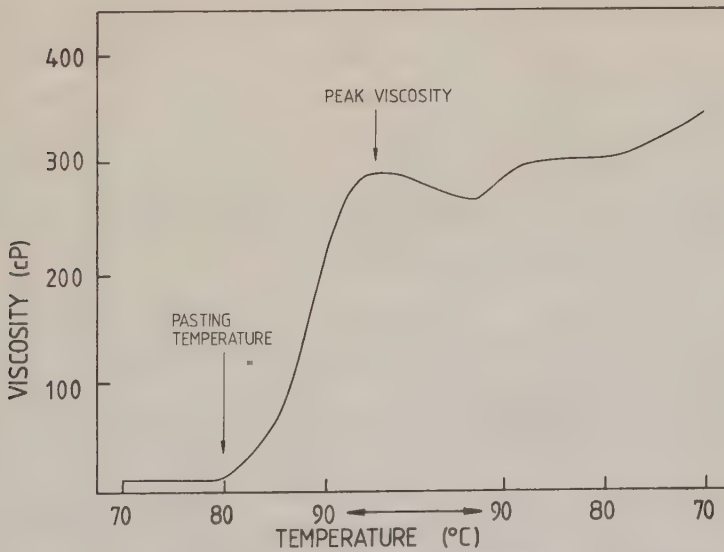


Fig. 9. The changes in viscosity when a wheat starch suspension (6% (w/w)) is heated at 1.5°C/min, held at about 90°C for 10 minutes, and then cooled (III).

close-packing of the swollen starch granules [31]. The flow behaviour was considered to be mainly determined by the volume fraction occupied by the granules. The role of the excluded amylose was explained by the fact that complete release of amylose is necessary to achieve maximum swelling of the starch granules. The importance of intergranular interactions, and the viscoelasticity and compressibility of the granules themselves for the rheological behaviour of gelatinized starch suspensions was also stressed [31].

The importance of the excluded material for the rheological behaviour of starch was demonstrated by Miller et al. [35]. Using a combination of amylograph, light microscope and SEM they found that the maximum viscosity of a wheat starch suspension heated in an excess of water occurred after most of the granule swelling ceased, and the increase in viscosity was found to be related to the material released from the starch granules. This material was, after freeze-drying, observed to be a network in both light microscope and SEM, and it stained blue with iodine (thus indicating amylose) whereas the starch granules stained red or magenta (thus indicating amylopectin). They also found that viscosity did not develop when no network was observed.

A stress-relaxation method has been used to investigate the rheological behaviour of starch gels containing 20-70% (w/w) starch (V). At the lowest starch concentration (20%) the water content is sufficient for complete gelatinization (I) whereas at the higher starch concentrations the second thermal transition might influence the properties of the final starch gel. The stress relaxation method used was developed for wheat dough and gluten [36,37] and was easily adapted for measurements on starch gels.

Since it was not possible to measure the stress-relaxation during the heating of the starch-water mixture, a heating sequence was simulated by heating the mixtures to different temperatures, allowing them to cool and then performing the stress-relaxation measurement. For each actual temperature a freshly prepared starch-water mixture was used. The relaxation modulus (G) and the half relaxation time ($T_{1/2}$) were calculated. These parameters are explained in Fig. 10, where examples of stress-relaxation curves are given. The G - and $T_{1/2}$ -values measured for wheat starch gels of different water contents as a function of heating temperature are given in Fig. 11. When these curves were compared to the corresponding DSC-thermograms (V) it was found that the maximum rigidity was obtained after the gelatinization transition (transition I) was completed. When excess water was present, no further change in G with tempe-

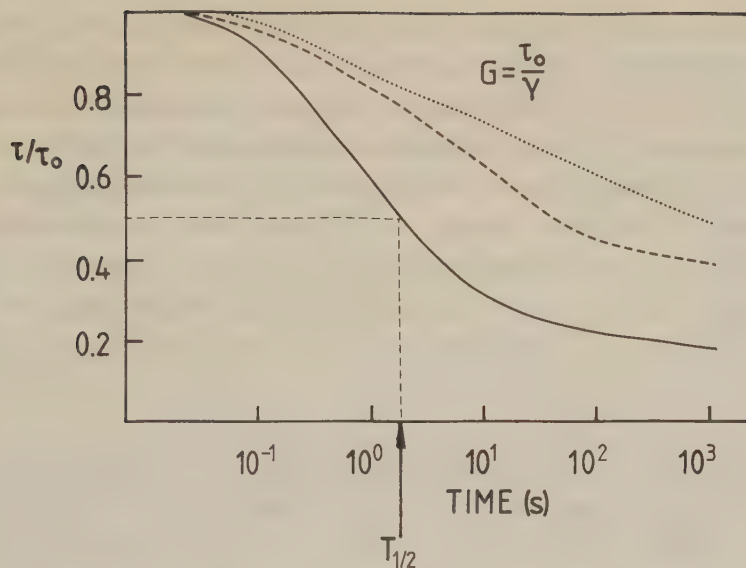


Fig. 10. Stress-relaxation curves for wheat starch gels (50% (w/w) water) heated to 68.5°C (—), 75°C (---) and 90°C (.....), respectively. $T_{1/2}$ is the time for the relative stress to decrease to half the initial value, and G is obtained by dividing the stress at zero time (τ_0) with the shear strain (γ). In this experiment the shear strain was 0.1 (V).

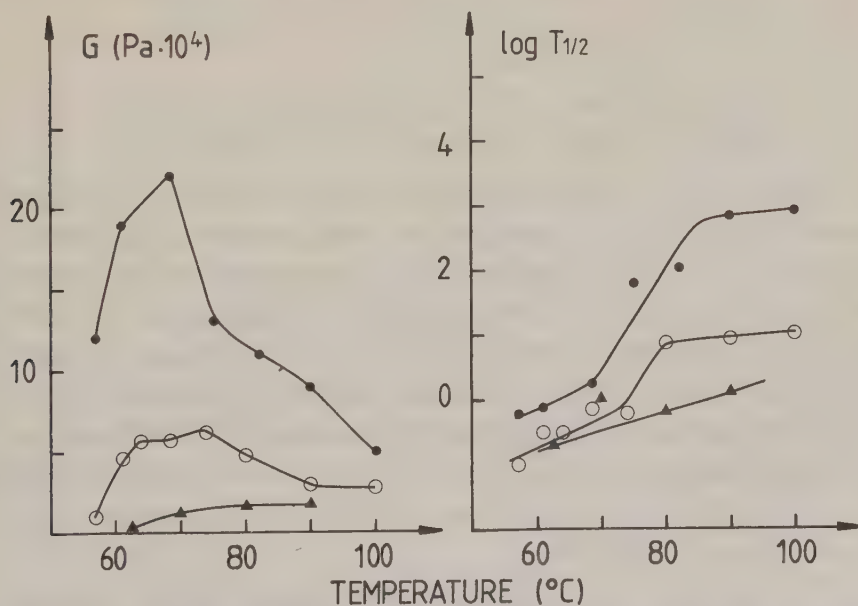


Fig. 11. Relaxation modulus (G) and half relaxation time ($T_{1/2}$) for wheat starch gels as a function of heating temperature (V).

- 50% (w/w) water, $\gamma = 0.1$
- 60% (w/w) water, $\gamma = 0.3$
- ▲—▲ 80% (w/w) water, $\gamma = 0.3$

perature was observed, whereas at lower water contents, G decreased. The second thermal transition might thus be interpreted as a process which makes the starch granules softer. This is consistent with the conception of the second thermal transition as a diluent dependant melting process: the rigidity of the starch granules decreases when the remaining crystallites disappear. At high water content $T_{\frac{1}{2}}$ increased slightly with temperature during the whole temperature interval investigated, whereas at limited water contents, a steep increase in $T_{\frac{1}{2}}$ occurred during the second transition. From the curve of relative stress *versus* time it was evident that no gel was formed when the water content was only 30% (V).

A starch gel might be described as a system consisting of highly swollen folded (and after severe heat treatment even fragmented) starch granules in a continuous aqueous phase of mainly amylose, which is released from the granules. In other words, the starch gel could be regarded as a composite material composed of a dispersed phase of starch granules in a continuous amylose solution.

The viscoelastic properties of the starch gel thus depend on:

- 1) the volume fraction occupied by the dispersed phase,
- 2) the rigidity of the dispersed phase,
- 3) the viscoelasticity of the continuous phase,
- 4) the adhesion between the dispersed and continuous phases.

Several factors known to influence the rheological properties of a starch gel can be explained by their influence on the above parameters. For example, a substance which retards the swelling of the starch granules may influence the rigidity of the starch granules and the volume fraction occupied by the granules. It may also indirectly influence the viscoelasticity of the continuous phase (due to a lesser amount of released amylose). Furthermore, it may affect the adhesion between the phases. The dependence of the properties of a starch gel on the preparation procedure (cf. ref. [32]) could be discussed in the same way, since the preparation procedure could be expected to influence the release of amylose.

The starch granule size distribution was found to influence the dynamic rigidity and viscosity at low starch concentrations [33,34]. In the case of wheat-starch gels containing 60% (w/w) water, an increase in the relative number of small granules caused $T_{\frac{1}{2}}$ to increase from 2.5 s to 7.5 s at 80°C

(V). The G-values were higher for this gel in the temperature interval of 60-80°C, whereas for gels heated to temperatures above 80°C, the G-values were independent of the granule size distribution. The differences in rheological properties between starch samples from different wheat varieties might to some extent be explained by the starch granule size distribution.

3.3 Influence of Lipids on Gelatinization

3.3.1 Lipids in Wheat Starch

The lipids in wheat starch can be divided into two groups (III):

1. Lipids on the starch granule surface ("surface lipids"), which may originate from the membrane surrounding the starch granule in the amyloplast. These lipids can be extracted by isopropanol (III), and thin layer chromatography (TLC) shows that they are rich in digalactosyl diglycerides and non-polar lipids.
2. Lipids inside the starch granules ("internal lipids"), which are present as the amylose-lipid inclusion complex. To extract these lipids water-saturated butanol at 100°C must be used [38]. Some of these lipids (probably those in the outer part of the starch granules) can be removed at room temperature, i.e. without gelatinization of the starch (III), and TLC shows that they are rich in mono-acyl lipids such as lysolecithin.

The amount and composition of the starch lipids in wheat and other cereals have been analyzed by Acker and Schmitz [6] and by Morrison [38]. For starch washed from a spring wheat, the total amount of lipids present was reported to be 1101 mg/100 g starch and these lipids consisted of 9% non-polar lipids (6% free fatty acids), 5% glycolipids and 86% phospholipids [38]. As for the nonstarch lipid content in the same wheat flour, the phospholipids amounted to only about 15%. The fatty acids in starch lipids are composed mainly of 16:0 and 18:2 and among the phospholipids, these fatty acids make up almost 90% of the total content. The composition of the phospholipids is given in Table 2. About 84% of the phospholipids consists of lysolecithin.

The amount of surface lipids as well as the amount of extractable internal lipids were found to differ between wheat varieties (III). The lipid content of the small and large granules from the same wheat variety (*Amy*) was also found to differ (VIII). The enthalpy of the amylose-lipid transition (ΔH_{III}) was used as a measure of the lipid content, and it was found that

TABLE 2
Composition of phospholipids in starch washed
from spring wheat flour [38]

Lipid	mg/100 g d.b.
Total phospholipid	955
Phosphatidyl ethanolamine + phosphatidyl glycerol	8
Phosphatidyl choline	36
Lysophosphatidyl ethanolamine + lysophosphatidyl glycerol	77
Lysophosphatidyl choline	807
Lysophosphatidyl serine + lysophosphatidyl inositol	27

ΔH_{III} of the small granules was 2-3 times greater than the values of the large granules (when compared on a weight basis).

The amylose in the amylose-lipid complex is less liable to undergo degradation. The degradation of complexed amylose with α -amylase (in vitro) is thus slower than the degradation of free amylose [39,40]. It has also been observed that the remaining starch in partly degraded A-granules has a higher phospholipid content than the undegraded starch [41]. The presence of the amylose-lipid complex lowers the activity of α -amylase, β -amylase, phosphorylase and branching enzyme [39]. The activity of the phosphorylase is affected more in the degradation of amylose than in the synthesis, whereas the activity of the enzyme synthetase is not affected by the amylose-lipid complex [39]. Based on these observations, Vieweg and de Fekete [39] suggested that the presence of amylose-lipid inclusion complexes might govern the amylose:amylopectin ratio in cereal starches, since the amylose in the complex is protected against degradation and branching, whereas elongation of the amylose still is possible.

This hypothesis is supported by the fact that during the development and maturation of barley the amount of lysolecithin increases in parallel with an increase in the relative amount of amylose [42]. There is also an increase in the amount of amylose-lipid complexes (observed as an increase in ΔH_{III}) during the development of wheat, rye and barley [43]. High-amylose varieties of maize [7] and pea [44] are known to be rich in lipids, whereas

the corresponding high-amylopectin varieties are almost free of lipids, and the normal varieties have a lipid content somewhere in between.

The amylose-lipid complex has also been suggested to promote the helix formation of the newly synthesized amylose and thus facilitate the ordered stacking of helices within the granule [45].

3.3.2 The Amylose-Lipid Inclusion Complex

The formation of the amylose-lipid complex mentioned in previous sections is a consequence of the ability of amylose to form a helical chain with a hydrophobic cavity. The helical amylose conformation is formed in the presence of compounds which can be accommodated into the helical cave; examples of these compounds include iodine [1], 1-butanol and various other organic molecules containing aliphatic chains (e.g. mono-acyl lipids) [46,47]. The best known complex is the amylose-iodine complex, the brilliant blue colour of which was discovered already in 1814 [48]. The ability of amylose to form complexes with surface active agents used in the food industry has been investigated by Osman et al. [49] and more recently by Krog [50].

The structure of V-amylose has been determined from X-ray diffraction analyses of dry (V_a -) and wet (V_h -) amylose-1-butanol complexes [51] and of the amylose-fatty acid complex [52]. The helical turn contains six glucose units and the unit cell is orthorhombic. The dimensions of these complexes are given in Table 3. The structure of the monostearin-amylose complex as determined by a Raman spectroscopy study is illustrated in Fig. 12 [53]. The hydrocarbon chain of monostearin was shown to be extended and ordered in the monostearin-amylose complex in a way similar to that in the crystalline state. The chain occupies three turn of the helix.

TABLE 3

The dimensions of the orthorhombic unit cell of V-amylose [51,52]

Complex	a_0 (Å)	b_0 (Å)	c_0 (Å)
1-Butanol (V_a)	13.0	22.5	7.90
1-Butanol (V_h)	13.65	23.70	8.05
Fatty acid (dry)	13.0	23.0	8.05
Fatty acid (wet)	13.7	23.8	8.05

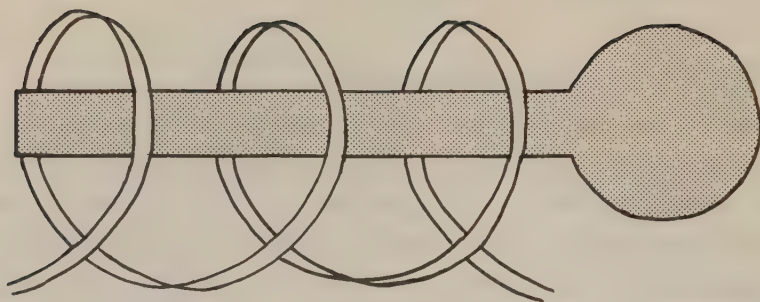


Fig. 12. The monostearin-amylose inclusion complex [53].

Only amylose, not amylopectin, is generally regarded as being capable of complex formation. When monostearin is added to a solution of amylopectin no precipitate is formed [50] and no thermal transition on the DSC-thermogram has been observed when amylopectin is heated and cooled in the presence of lysolecithin [23]. However, the possibility of amylopectin-lipid complex formation should not be totally ruled out, since interaction between iodine and amylopectin is known to take place [1]. The effects of polar lipids on the retrogradation of starch indicate that some kind of interaction between amylopectin and the lipids occurs (VII). The degree of polymerization of the amylose is known to influence the complex formation between amylose and iodine [1] and similar effects should also be expected in the case of complex formation between amylose and polar lipids. It has been observed [54] that the enthalpy of the high-temperature endotherm depends on whether amylose of high or low molecular weight is used.

For efficient complex formation the lipid monomer solubility in water should be as high as possible. Polar lipids are either soluble in micellar form, as e.g. lysolecithin, or dispersible in water in the form of crystals, liquid

crystals or L2-phases. The best condition for complex formation with a lipid dispersion in water is obtained when the lipid forms a lamellar liquid crystalline phase [13,55]. Formation of amylose-lipid complexes has been demonstrated in the case of lipids with a single acyl chain per molecule, and of various lipids examined, the distilled monoglycerides proved to be the most effective, as estimated from iodine displacement ability [50]. When the ability of 1-monoglycerides to complex amylose was compared at a temperature of 60°C it was found that the most effective chain length is C-14, with C-12 and C-16 almost as effective [50]. At this temperature, the lamellar liquid crystalline phase exists for monoglycerides with chain lengths extending from C-12 to C-18 [55]. With increasing unsaturation in the fatty acid chain, the amylose complexing ability decreases [50], which might be an effect of steric requirements when a *cis*-double bond is accommodated into the helical cavity.

The thermal properties of amylose-lipid complexes of the following types will now be discussed:

- A. Amylose-lipid complexes occurring in native wheat starch (III and VIII).
- B. Amylose-lipid complexes formed by addition of lipids to an amylose solution.
- C. Amylose-lipid complexes formed during the gelatinization of starch in the presence of added polar lipids (IV and VII).

A. Amylose-Lipid Complexes in Native Starch

In the previous sections the mono-acyl lipids present in native wheat starch (Table 2) are considered to be present in the form of the amylose-lipid inclusion complex. This is based on the work by Acker and Becker [7], and as evidence for the existence of the amylose-lipid inclusion complex in native starch, they referred to the difficulty in extracting the cereal starch lipids, the comparatively low iodine binding capacity of undefatted wheat starch, and to the protection of starch-bound lysolecithin against enzymatic degradation as well as against oxidation. The V-pattern, however, is not observed for the native starch until after gelatinization [56]. It should be pointed out that the helical amylose conformation of the lipid or iodine complex does not directly correspond to the V-amylose crystalline structure, which can be identified by means of X-ray diffraction. A requirement for the diffraction effect is that the size of the ordered regions (crystallites) is larger than about 10^{-5} cm.

The transition observed on the DSC-thermogram is reversible as illustrated in Fig. 5. This transition is considered to correspond to a melting of the V-structure of amylose [23]. The temperature of the exothermic transition during cooling is lower than the temperature (T_{III}) of the endothermic transition during heating. T_{III} was found to depend on the water content (I), whereas no differences in T_{III} between wheat varieties were observed (III). The transition enthalpy (ΔH_{III}) seemed to be independent of the water content in the range of water to starch from 3:1 to 1:1 (VIII). ΔH_{III} is of course dependent on the amount of complex present, and it has been used as a measurement of amylose content [54] and of lipid content (VIII).

B. Amylose-Lipid Complexes Formed by the Addition of Lipids to a Solution of Amylose

After a certain amount of, e.g., a micellar solution of lysolecithin is added to a solution of amylose, the amylose-lipid complex precipitates. This precipitate can be separated from the solution and used directly for DSC-measurements (method a), or dried at room temperature and rehydrated before the DSC-measurements (method b). The complex formation has been followed by Kugimiya et al. [23] after mixing amylose, lysolecithin and water in a DSC-sample pan (method c), and they also added lipids dissolved in ethanol to the amylose solution (method d). T_{III} -values for some amylose-lipid complexes are given in Table 4, and the values are seen to vary with different lipid compounds as well as with the preparation method. The DSC-thermograms for the complexes formed by saturated (monopalmitate) and unsaturated (distilled sunflower oil) monoglycerides are given in Fig. 13. The low transition temperature (93.8°C) and the broad and flat appearance of the DSC-peak for the unsaturated monoglyceride suggest a less ordered structure. The macroscopic appearance was a semi-transparent "gel" in contrast to the white, dense complex formed by the saturated monoglyceride. The higher transition temperature (98.0°C) and the narrow and sharp appearance of the DSC-peak for the latter complex suggest a more solid-like structure. The complex formed by the saturated monoglyceride was found to contain 92.9% water, whereas the complex formed by the unsaturated monoglycerides contained 96.5% water. The amount of "bound water" (i.e. water which did not freeze when the samples were cooled to -53°C) was determined by DSC, and the amount of bound water per gram dry complex was 0.7 g in the case of the saturated monoglyceride and 1.0 g in the case of unsaturated monoglycerides.

TABLE 4
Transition temperatures (T_{III}) for amylose-lipid complexes

Lipid	Method ¹	T_{III} ($^{\circ}\text{C}$) ²	Reference
Lysolecithin			
egg lysolecithin	c	104.7 ± 0.4	[54]
synthetic, palmitoyl	c	103.6	[54]
egg lysolecithin	b	107.7	3)
Palmitic acid	c	no endotherm observed	[23]
	d	97 and 104	[23]
Monoglycerides			
saturated	a	98.0 ± 0.8	[57]
unsaturated	a	93.8 ± 0.7	[57]

1) Methods a, b, c and d, see text

2) See Figs. 5 and 13

3) DSC-measurements as described in e.g. (IV)

C. Amylose-Lipid Complexes Formed Between Added Lipids and Gelatinizing Starch

Potato starch, which naturally contains only trace amounts of lipids [58], is especially suited for studying the effects of added lipids. When the polar lipids (preferably in the lamellar liquid crystalline phase) are added to starch, the complex formation occurs during the gelatinization, i.e. upon the release of amylose molecules from the granules. The complex should be expected to form an insoluble surface layer outside the granules. The technical significance of such an encapsulation will be discussed in section 3.3.3. Some surfactants (sodium dodecyl sulphate, and cetyltrimethylammonium bromide) seem to solubilize the amylose molecules from the starch granules at temperatures below the gelatinization temperature, since they increase the swelling [59] and also decrease the gelatinization temperature [60]. This effect might also be obtained when a mixture of potato starch and monoglycerides is held at temperatures below the gelatinization temperature over a long period of time [61]. ΔH_{III} - and T_{III} -values for a number of cases where lipids have been added to potato starch are given in Table 5. ΔH_{III} increases with increasing amount of added lipid, due to the greater number of amylose-lipid complexes formed, but also T_{III} increases. This might be due to a higher order of packing in the V-structure of the formed complexes when the amylose

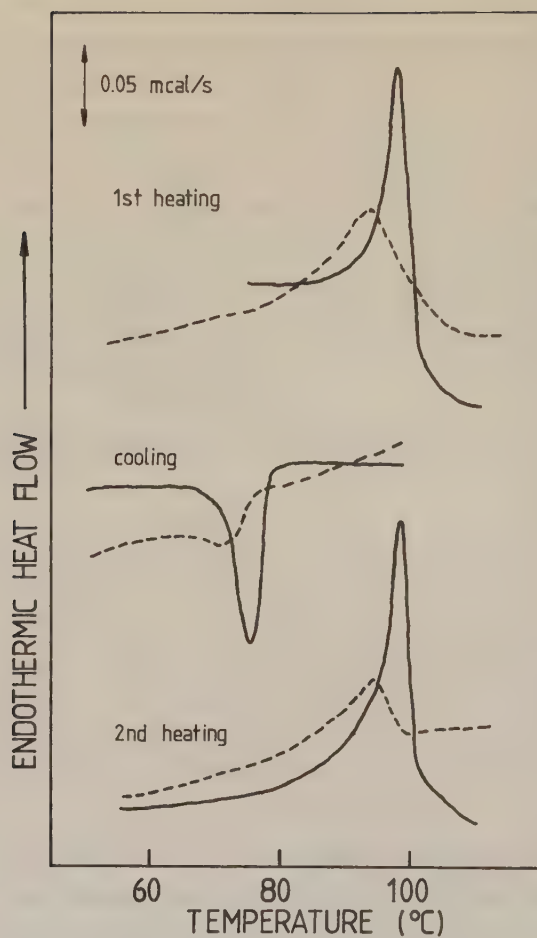


Fig. 13. DSC-thermograms of amylose-lipid complexes with monopalmitate (—) and distilled sunflower oil (---), respectively.

contains more lipids. T_{III} -values for the lysolecithin complexes are higher than the T_{III} -values for the monoglyceride complexes in accordance with the results reported in Table 4.

TABLE 5

Transition enthalpies (ΔH_{III}) and transition temperatures (T_{III}) for amylose-lipid complexes formed during gelatinization of potato starch in the presence of lipids

Lipid	Amount of lipid (g/g starch)	ΔH_{III} ¹ (cal/g)	T_{III} ¹ (°C)	Reference
Lysolecithin	0.03	1.0	-	[54]
	0.10	1.22	104.9	[54]
Monoglycerides	0.006	0.16	96.9	(IV)
	0.024	0.50	99.7	(IV)
SDS ²	0.003	0.12	98.3	3)
	0.05	1.22	105.3	3)

1) See Fig. 5

2) SDS = Sodium dodecyl sulphate

3) DSC performed at the same conditions as in (IV). Potato starch was mixed with a SDS-solution (5 mM or 100 mM) in the proportions 65% potato starch to 35% SDS-solution.

3.3.3 Gelatinization in the Presence of Lipids

The gelatinization model adopted in the previous sections involves release of amylose from the starch granule, which in turn results in uptake of water. It was also suggested that the amylose-lipid complexes formed are "insoluble" and form a barrier at the surface of the starch granule. The lysolecithin content in wheat starch [6] is sufficient for complexing about 25% of the total amount of amylose. It should thus be expected that the presence of amylose-lipid complexes will influence the gelatinization process. This hypothesis was tested for the following starches:

1. Two wheat starch varieties with differing amounts of extractable lipids (III).
2. Potato starch, native and coated with monoglycerides (IV).

Viscosity measurements were used to characterize the two wheat varieties: one with a large amount of lipids (*Amy*), and the other with a lower amount (*Maris Huntsman*) (III). *Amy* showed the highest pasting temperature (see Fig. 9) and the lowest peak viscosity. Removal of lipids caused the pasting temperature to decrease and the peak viscosity to increase. This was also

the case for *Maris Huntsman*, although the influence on the peak viscosity was less.

The coating of potato starch granules with monoglycerides (IV) was performed to achieve optimal conditions for complex formation during gelatinization, according to the discussion in section 3.3.2 and the presence of amylose-lipid complexes was demonstrated by the high-temperature endotherm on the DSC-thermogram. The formation of amylose-lipid complexes delayed the swelling of the starch granules (IV). The gel volume was also found to decrease, and at the highest level of added lipids (2.8% (w/w)) the gel volume was about the same as for a wheat starch gel. Similar effects of added lipids were observed when wheat and cassava starches were gelatinized in the presence of monoglycerides [62]. It is also known that the addition of lipids to potato starch causes its pasting properties to resemble those of wheat starch [63,64]. These effects of lipids on the swelling and pasting behaviour of starch are in accordance with the discussion above, i.e. a decreased release of amylose causes a decreased uptake of water, and thus the rheological behaviour changes. Differences in rheological properties between wheat varieties might thus depend on the amylose-lipid complex present.

The transition enthalpies (ΔH_I and ΔH_{II}) differed between the two extreme wheat starch varieties examined (III). The starch with lowest ΔH_I -value (*Amy*) contained the highest amount of adhering surface lipids as well as of complex bound lipids. The low ΔH_I -values were attributed to an inhibition of gelatinization due to the amylose-lipid complex (a delayed amylose and water diffusion), since the difference between the varieties disappeared when the lipids were removed (III). The minimum water content necessary for gelatinization to occur was higher for the starch from *Amy*. Moreover, the ΔH_{II} -values of *Amy* were also lower. The transition temperatures seemed to be less influenced by the presence of lipids, although the gelatinization started at a lower temperature for *Maris Huntsman*. A decrease in ΔH_I and an increase in T_I were observed when a mixture of wheat starch and gluten (VII) was gelatinized in presence of sodium stearyl lactylate (SSL).

The thermal properties of potato starch coated with lipids were measured in excess water (IV). The starch was freeze-dried prior to the DSC-measurements and a sample of potato starch freeze-dried without added lipids was also included in the measurements. The freeze-drying caused T_I to decrease from 66 to 59°C, and ΔH_I from 5.2 to 3.9 cal/g starch. No significant

effects on T_I and ΔH_I were obtained when lipids were added.

Amylose-lipid complexes in wheat starch are located within the starch granules, and thus prevent the release of the complexed amylose during the gelatinization. This may affect the water content of the starch granules according to the model which assumes amylose leakage to be a requirement for water uptake, and thus also ΔH_I according to the discussion in section 3.1. In potato starch, on the other hand, the amylose-lipid complex is formed at the starch granule surface when the amylose molecules are released during gelatinization. When excess water is present, there is enough water both to penetrate the starch granule and to hydrate the amylose-lipid complexes on the granule surface. Thus ΔH_I is not affected. The effect of the "insoluble" barrier on the starch granule surface may also depend on the kinetics of gelatinization. The influence of lipids on ΔH_I in excess water was independent of the scanning rate (IV), but at lower water contents, such kinetic effects can be more pronounced (cf. VII).

3.4 Influence of Gluten on Gelatinization

The gluten used (II) was prepared from wheat flour (*Amy*) in an automatic gluten washer, sealed in a sample pan, and heated in the calorimeter. The transition enthalpies in gluten (0.03 - 0.06 cal/g gluten) were very small compared to enthalpies for denaturation of proteins (3 - 10 cal/g for several proteins [65]). None of the peaks were obtained upon a second heating (II). The small endothermic flows observed indicate that the gluten proteins are only marginally affected during baking, and these small transition enthalpies make it possible to use the DSC to study gelatinization of starch in the presence of gluten.

The addition of gluten caused the gelatinization enthalpy (ΔH_I) to decrease and the gelatinization temperature (T_I) to increase in proportion to the amount of gluten added (VI). T_{II} also increased whereas ΔH_{II} was almost constant. The effect of gluten could, in accordance with the discussion in section 3.1, be interpreted as resulting from its influence on the amount of water available for the starch. This amount was found to increase during heating, whereas the percentage of the total amount of water associated with gluten decreased from 16% during transition I (Fig. 5) to 11% during transition II.

4. RETROGRADATION

The starch gel is by no means a system in thermodynamic equilibrium, and changes in the gel structure start immediately after cooling. Changes characteristic for the ageing of a starch gel involve increased opaque appearance and formation of a more rigid gel net-work. Furthermore, the iodine affinity of the starch molecules decreases as well as the digestibility by hydrolyzing enzymes or acids. The retrogradation is basically a crystallization process, which is shown by changes in the X-ray diffraction pattern. In cereal starches, the A-pattern is lost during gelatinization, and a V-pattern is obtained due to the amylose-lipid complex. With time the B-pattern will develop superimposed on the V-pattern [66]. Sarko and Wu [5] proposed that the retrogradation is due to cross-linking of chains by double helical "gel junction zones", and that the retrogradation might be prevented if the double helix formation is avoided, for example by complexing to give the V-helix. When the maximum crystallinity in a starch gel is obtained, only about 15% of the starch is in the crystalline form [67].

The crystallization has been followed directly by means of DTA [68] and DSC (VII), [69,70] and indirectly by rheological methods (cf. ref. [67]).

The Avrami model has been used to interpret the results obtained (e.g. ref. [68]). According to this model, the fraction of uncrystallized material (θ) depends on time (t) as expressed in the Avrami equation:

$$\theta = \exp (-kt^n)$$

where k is a rate constant, and n (the Avrami exponent) is an integer which describes the nucleation and growth of crystals. For starch gels the exponent has been claimed to be 1 (see e.g. ref. [68]), corresponding to rodlike growth from instantaneous nuclei.

4.1 Retrogradation Measured by Differential Scanning Calorimetry

Examples of DSC-thermograms for reheating samples of stored bread crumb, starch gel, amylopectin, and amylose are given in Fig. 14. In the DSC-method, thus the melting of amylopectin (Fig. 14; [22]) is measured during reheating of a stored starch gel, and the enthalpy of this melting process (ΔH_c) is proportional to the amount of crystallized amylopectin.

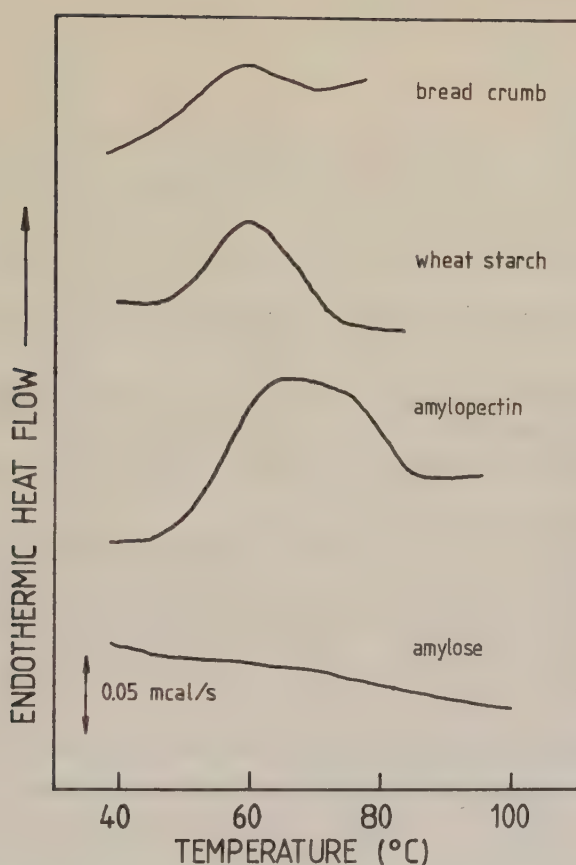


Fig. 14. DSC-thermograms of reheating samples of amylose, amylopectin, wheat starch and bread crumb stored for seven days at room temperature. Amylose, amylopectin and wheat starch were mixed with water (1:1) and heated to 100°C in the DSC before storage.

For the starch gels investigated in (VII) it was found that the greatest change in ΔH_c occurred during the first day of storage. Analysis of these results according to the Avrami-model gave a rate constant of 0.65 days^{-1} and an Avrami-exponent less than unity (0.5). Fractional n values have been observed earlier [69-71], and in these cases the n values do not have unambiguous meaning [68]. The water content of the starch gels was found to influence the starch crystallization (VII; [69]), and a maximum in crystallization was obtained at about 0.8 g water/g starch (VII).

4.2 Rheological Changes in an Ageing Starch Gel

The retrogradation increases the rigidity of the starch gel, and measurements of the increased firmness by some kind of compression test is the most common method used for following the ageing of starch gels and bread crumb (see e.g. ref. [67]). A slight thixotropic behaviour was observed when the flow curve for gelatinized wheat starch gel was obtained at 25°C, whereas the behaviour was non-thixotropic at temperatures above 50°C [32].

The ageing of the starch gels was also found to influence the stress relaxation measurements. In the experiments discussed in section 3.2, the time between the heating and the onset of the stress relaxation measurements was fixed at 40 minutes. When G and $T_{\frac{1}{2}}$ were measured after longer periods of time, the results in Table 6 were obtained. The value of G increased with time, whereas there was a drastic decrease in $T_{\frac{1}{2}}$ (from 735 to 5.7 s during the first day of storage). In the composite material (a model of starch gel used in section 3.2) increased G -values and decreased $T_{\frac{1}{2}}$ -values are measured when the adhesion between the continuous and dispersed phases decreases.

TABLE 6

Relaxation modulus (G), half relaxation time ($T_{\frac{1}{2}}$), and ΔH_C for starch-water mixtures containing 50% (w/w) water heated to 90°C and stored for 1, 3 or 7 days

Age	G^1 (Pa)	$T_{\frac{1}{2}}^1$ (s)	ΔH_C^2 (cal/g starch)
40 minutes	8.55×10^4	735	0.00
1 day	9.86×10^4	5.7	0.50 ± 0.18
3 days	1.46×10^5	2.6	1.30 ± 0.05
7 days	1.63×10^5	2.5	1.76 ± 0.11

1) Measured as described in (V)

2) Measured as described in (VII)

Also the dynamic rigidity of wheat starch gels increased during ageing [72]. The limiting value of the dynamic rigidity was obtained after about 60 hours for wheat starch gels stored at 20°C. This corresponds to the increase in relaxation modulus, which increased at a much slower rate after the third day of storage (see Table 6). It thus seems that the greatest change in half

relaxation time occurs during the first day of storage, whereas the increase in crystallinity and the increase in rigidity of the gel continue for a longer time (Table 6).

4.3 Influence of Gluten and Lipids on Retrogradation

It was found that the presence of gluten in a starch gel delayed the crystallization (VII), which was related to the lower amount of water available for the starch. Moreover, some other factors besides the available water influence the starch crystallization. The film of gluten on the surface of the starch granules might inhibit, to some extent, the release of amylose during the gelatinization of the starch. The influence of the wheat variety on the starch crystallization was also examined and it was found that the gluten variety did not influence ΔH_C , whereas the starch variety did.

The influence of surfactants on the starch crystallization was investigated (VII) and the formation of amylose-lipid complexes was checked by heating the stored gels to temperatures high enough for the phase transition of the amylose-lipid complex to occur. The following observations were made using SSL as surfactant:

1. The surfactant decreased ΔH_I and increased T_I during gelatinization in proportion to the amount added. The starch gelatinization was thus inhibited in the way discussed in section 3.3.3.
2. The surfactant decreased ΔH_C at all storage times investigated (1, 3 and 7 days). This decrease in ΔH_C was independent of the amount of SSL added.
3. Analysis of the results according to the Avrami-model gave an Avrami-exponent less than unity.
4. The added surfactant formed the amylose complex, an effect which was observed as an increased $\Delta H_{I'I}$ -value.

Surfactants have been used for a long time in the baking industry to increase the keeping qualities of bread [67], and one of the reasons for the good effect of the surfactants in this respect has been claimed to be the amylose-lipid complex formation [73]. According to the present results, however, the ΔH_C -values, which emerge from the melting of crystallized amylopectin (see Fig. 14) are decreased by the addition of SSL. There is no direct relation between the formation of amylose-lipid complexes and ΔH_C , since new complexes are formed without affecting the ΔH_C -values. As discussed above (see section 3.3.2), the amylose-lipid complex can form different types of gel-like phases

with differing water-holding capacity, and this might be of importance as regards the role of surfactants in delaying the starch crystallization. In this way the amylose-lipid complexes could influence the amount of water available to the starch.

5. STARCH IN DOUGH AND BREAD

In the previous sections, the behaviour of starch in simple model systems with limited amounts of water was discussed. In order to make the model system more like doughs and breads, lipids and gluten were introduced, and the interaction between these components and starch was studied. In this section the implications of these results with regard to the more complex system of dough and bread will be discussed.

5.1 Dough Mixing and Fermentation

From a physical-structural point of view, dough is a multi-phase system with water as the continuous phase, and protein aggregates, starch granules, air cells, and yeast cells as dispersed phases. The colloidal structure of wheat flour dough has been extensively analyzed by Carlson [74]. According to his model of the structure, which will be used here, the aqueous protein matrix ('gluten') is regarded as the continuous phase.

During dough making, the surface properties of the starch granules are of importance, since adhesion between gluten and starch determines the dispersibility of the starch granules (III). The small granules make possible a closer packing of the starch granules (V), and the small granules seem to adhere more strongly to gluten (indicated by the difficulties in washing gluten free from this fraction of starch).

The water is distributed between starch and non-starch components, and this distribution influences the rheological properties of the dough. The water distribution is mainly determined by starch and gluten, as in the simplified model system used in (VI). The amount of water which will be available for the starch during the gelatinization should therefore be expected to depend on the starch variety (c.f. III and VI), and also on the amount of damaged starch. The gluten variety, however, was found to have less influence. Also pentosans could be expected to have an influence on the water distribution due to their efficient water-holding capacity [75]. Furthermore, added ingredients such as salt and sugar affect the distribution of the water

between the components. Thus the amount of water associated with the continuous and dispersed phases, respectively, will differ, and due to the low water content, there will be competition for the water.

During dough making and fermentation, the starch granules are partly degraded by amylases. The amount of damaged starch is crucial, and furthermore, the amount of starch bound lipids could be important since the formation of complexes between amylose and lipids might protect the amylose against enzymatic breakdown.

5.2 Baking

The extent of starch gelatinization during the baking process depends on the amount of water available for the starch (I), and this also affects the final texture of the crumb (cf. V).

The presence of gluten retards the starch gelatinization to some extent (VI) but also the added ingredients have similar effects [76], (VII). It is natural to regard the effect of the additional dough components on the extent of starch gelatinization as due either to competition for water or to formation of a surface film on the starch granules, producing a barrier against the transport of water into the granules.

The extent of starch gelatinization and as a consequence of this, the final crumb texture may vary with wheat variety (VI). Thus differences in the amount of amylose-lipid complex will influence the gelatinization properties (III), and these complexes may also be of importance during the temperature interval when the α -amylase activity is at an optimum. Furthermore, the relative proportion of small granules influences the gelatinization temperature (VIII), as well as the rheological properties of the starch gel (V), thus indicating that the proportion of small granules is important even for the final baking result.

During the thermal transitions (I) the starch granules are assumed to become softer (V). This should be expected to affect the properties of the gas-liquid interface, since the starch granules must be flexible enough to be properly oriented at this interface without causing rupture and destroying the gas-holding capacity. The starch granules seem to be stretched and elongated in this process [77].

The diffusion of amylose from the granules during the gelatinization, and the swelling and distortion of granules make contacts between granules possible, and it has been suggested that the starch gel formed could even constitute a continuous structure in bread [78]. From SEM-pictures of bread crumb, on the other hand, it has been concluded that the gluten phase is continuous [79,80] with the more or less gelatinized starch dispersed.

5.3 Staling

The staling of bread is a complex phenomenon, and the two dominating processes which take place in the bread crumb will be considered here, i.e. retrogradation (crystallization) of starch and transport (redistribution) of water.

The literature on the mechanisms behind staling, the methods for measuring staling, and the methods for delaying it has been thoroughly reviewed by Maga [67], and this paper illustrates just how controversial this field is.

The DSC-method gives a direct method for measuring the crystallization of starch. The peak obtained on the DSC-thermogram upon reheating stale bread emerges as a result of the melting of amylopectin ([22]; Fig. 14). These results support the idea that the retrogradation of amylopectin is responsible for most of the staling related to starch crystallization.

The role of water seems to be very important in the staling process. As gluten is at least partly a continuous phase in bread, a migration of water from gluten to starch, according to results reported by Willhoft [81] will strongly affect the rheological properties of the crumb. As observed in this work (VI), there is still water left in the gluten after the highest baking temperature has been reached, and the water content of the starch is lower than the amount that starch can absorb in excess water. There is thus a driving force for the starch to absorb more water. The function of many hydrocolloids, which increase the shelf-life of bread, could then be to compete with the starch for the available water, and thus increase the water content of the continuous phase. The starch crystallization has also been suggested to influence the water content of the continuous phase, depending on which crystal structure is formed [82]. A bread, which gave V- and A-structures, were softer than a bread which gave V- and B-structures, which was explained by the fact that the B-structure contains more water in the unit cell.

The increased firmness and crumbliness of bread during staling is thus a result of a drying out of the continuous phase, and a hardening of the starch granules due to crystallization. In section 4.2 (Table 6) it was shown that the relaxation modulus increased during the ageing of the starch gel, whereas the half relaxation time decreased. This was interpreted as a decreased adhesion between the continuous and dispersed phases, due to the crystallization process.

In this work it was found (VII) that the crystallization process is affected both by gluten and added lipids. The effect of the lipids on retrogradation has been analyzed [73], and it was proposed that amylose-complexing in the aqueous medium is the mechanism behind the crumb softening. An additional effect could be that the amylose complex, due to its insoluble character, will act as a barrier outside the granules, and therefore delay the transport of water from the gluten phase into the granules. The crystallization of starch was found to differ between wheat varieties (VII), and this might be a feature of wheat flour which should be taken into consideration, together with the baking performance, in the breeding of new wheat varieties.

6 SUMMARY

The aim of this work has been to contribute to the understanding of the role of starch in bread-making and in staling of bread. Gelatinization and retrogradation of starch in concentrated aqueous systems have therefore been studied using differential scanning calorimetry (DSC) and stress-relaxation technique.

An aqueous suspension of wheat starch exhibits three endothermic transitions. The high-temperature endotherm is due to an amylose-lipid complex whereas the other two endotherms are directly related to the gelatinization process. According to the thermal behaviour, it is natural to describe the gelatinization as a diluent-dependant melting of the crystalline regions in the starch granules. The low temperature endotherm emerges from a cooperative melting in excess water, and the second endotherm represents a kind of melting phenomenon when no excess water is present.

The rheological properties of concentrated wheat starch gels have been measured in stress-relaxation experiments, and on the basis of the results, the starch gel is described as a composite material with more or less swollen granules dispersed in an aqueous amylose solution.

Interaction between starch and gluten was also studied. Gluten itself is only marginally influenced by heat, i.e. very small transition enthalpies are observed by DSC. The presence of gluten was found to increase the gelatinization temperature and decrease the gelatinization enthalpy of wheat starch.

In studies of the interaction between starch and lipids it was found that the formation of amylose-lipid complexes results in a delay in the gelatinization process, as was obvious from decreased swelling of the starch granules, increased "pasting" temperature, reduction of viscosity, and decreased gelatinization enthalpy at low water contents. Differences between wheat starch varieties in this respect were observed.

The retrogradation of starch is reduced by the presence of gluten as well as lipids. Depending on the structure of the lipid, amylose-lipid complexes with different thermal properties and water-holding capacities can be formed, and these differences are proposed to be relevant for their anti-staling effect.

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I

EFFECT OF WATER CONTENT ON THE GELATINIZATION
OF WHEAT STARCH

by

A.-C. Eliasson

Effect of Water Content on the Gelatinization of Wheat Starch

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The influence of water content on the gelatinization of wheat starch was examined by differential scanning calorimetry. Three endothermic transitions were observed when starch was heated to 140°C with 35 to 80% (w/w) water. The temperature of the second and third endotherms and the enthalpies of the first and second endotherms vary with water content.

Die Wirkung des Wassergehaltes auf die Verkleisterung von Weizenstärke. Der Einfluß des Wassergehaltes auf die Verkleisterung von Weizenstärke wurde mit Hilfe der Differential-Raster-Kalorimetrie untersucht. Wenn Stärke mit 35 bis 80% (w/w) Wasser auf 140°C erhitzt wurde, konnten drei endotherme Übergänge beobachtet werden. Die Temperatur der zweiten und dritten Endothermen und der Enthalpien der ersten und zweiten Endothermen verändert sich mit dem Wassergehalt.

1 Introduction

One of the major functions of starch in breadmaking is to bring about a proper water balance [1]. As a consequence of the competition between the dough components for available water, the thermal gelatinization of starch occurs with limited amounts of water. Still there are few reports on the gelatinization of starch in such concentrated systems. Recently, differential scanning calorimetry (DSC) was used to study starch gelatinization over a wide range of starch to water ratios for potato starch [2] and for wheat starch [3]. The general features of the thermal transitions are different, however, in these both investigations. The aim of this study has been to elucidate whether there really are differences in the thermal behavior between wheat starch and potato starch or if the divergence can be assigned to the different experimental conditions in these two investigations.

2 Material and Methods

2.1 Starch

Starch from the Swedish spring wheat Amy was used throughout all experiments. The starch was prepared according to Meredith et al. [4] to avoid damage of the starch granules.

The moisture of the starch was analysed using the AACC-method 44-15 [5].

Starch and water were mixed in appropriate ratios and transferred to an aluminium sample pan (Perkin-Elmer No. 219-0062); 5-20 mg of the starch-water mixture were used.

2.2 Differential Scanning Calorimetry

The thermal transitions were measured by differential scanning calorimetry carried out on a Perkin-Elmer DSC-2.

An empty pan was used as reference. The experiments were carried out at a scanning rate of 10°C/min when the influence of water content was studied. Measurements were also performed at scanning rates of 5°C/min and 20°C/min to study the influence of scanning rate on the appearance of the endothermic transitions. Transition temperatures were defined as the intersection of the extrapolated lower temperature side and the extrapolated higher temperature side of the DSC peak. Calibration of the DSC and the calculation of the energies have been described earlier [6]. All reported values are the means of 3–4 independent replicates.

3 Results and Discussion

Three endothermic processes are observed on the DSC-thermogram when a starch-water-mixture with water content in the interval 35–80% (w/w) is heated to 140°C (Fig. 1). Donovan [2] observed two endothermic peaks when heating potato starch to 150°C. Wootton and Bamunuarachchi [3] did not report more than one endothermic peak when wheat starch was heated to 135°C, but no experimental curves were given.

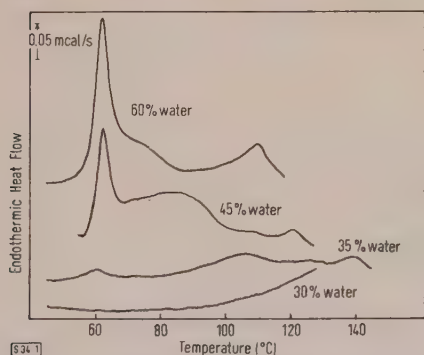


Figure 1. Examples of DSC-thermograms when wheat starch-water-mixtures are heated at a scanning rate of 10°C/min.

The temperature of the first endothermic transition does not vary significantly with water content (Fig. 2). The temperatures of the second and third transitions, however, are related to the water content (Fig. 2). When the water content decreases, the endotherms are shifted towards higher temperatures.

All three endothermic DSC-peaks are observed only at intermediate starch-to-water-ratios. When enough water is present, the second peak disappears. Figure 3 shows the dependence of the transition enthalpy on the water content for the first and the second transitions. The enthalpy of the third endotherm is not calculated because of the difficulty to draw an appropriate baseline in this region of the thermogram.

There is a linear relationship between the transition enthalpy of the first endotherm and the water content to a water content of 70% (w/w). More water seems not to affect the transition enthalpy but the variations of the enthalpies are strong in this region. Extrapolation of the enthalpy to zero gives the minimum water content necessary for gelatinization of wheat starch. According to Figure 3 this value is 33% (w/w), which is in good agreement with the value of 32% (w/w) observed by Wootton and Bamunuarachchi [3].

The enthalpy of the second transition reaches a maximum at a water content of 45% (w/w). The second peak in potato starch

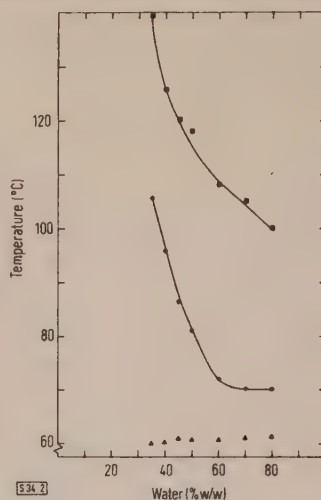


Figure 2. Dependence on water content of the transition temperatures for the three endotherms observed on the DSC-thermogram for wheat starch; $\blacktriangle$ = first endothermic peak; $\bullet$ = second endothermic peak; $\blacksquare$ = third endothermic peak.

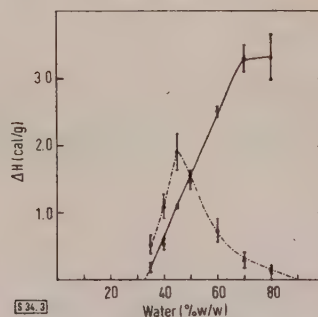


Figure 3. Dependence on water content of the transition enthalpies for the first and second endothermic transitions in wheat starch; $\bullet$ = first endothermic transition; $\blacktriangle$ = second endothermic transition.

reaches its maximum at a molar ratio of 4 H₂O/hexose unit, which corresponds to about 31% (w/w) water [2]. This is also the necessary water content for gelatinization. Different scanning rates do not explain the appearance of the second endothermic peak. Table 1 indicates that the ratio between the enthalpies of the two processes is independent of the scanning rate.

Table 1.
Influence of Scanning Rate on the Ratio Between the Enthalpies of the Endothermic Processes. Water Content: 50% (w/w).

Scanning rate (°C/min)	ΔH first peak
	ΔH second peak
20	1.3 ± 0.1
10	1.0 ± 0.2
5	1.1 ± 0.1

4 Conclusion

This investigation clearly shows that more than one transition occurs when heating wheat starch under conditions relevant to breadmaking. There are pronounced differences between wheat starch and potato starch. The endothermic transitions coexist at different water contents in wheat starch compared to potato starch. But the enthalpies of the first and second endotherms are depending on the water content in wheat starch as in potato starch.

This work will be continued by studying available water for starch in doughs made from different cereals.

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Thermal Stability of Wheat Gluten

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ABSTRACT

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The thermal behavior of gluten was studied with differential scanning calorimetry in the temperature range 30–115°C. Four endothermic peaks were registered. The peaks at 64.6 and 111.8°C were assigned to thermal transitions in starch, whereas the peaks at 88.4 and 101.4°C were assigned

to transitions in the gluten proteins. The apparent enthalpies of the protein transitions were, however, very small. These small enthalpies do not support the idea of protein denaturation as an important factor in the baking process.

The texture of bread is reputed to be the result of starch gelatinization and protein denaturation during the baking of fermented dough (Parker 1960, Ponte 1971). In the baking process, the temperature is raised from the fermentation temperature to about 95°C in the center of the bread. The influence of this thermal treatment on the different dough constituents is poorly understood. Starch has probably been examined in most detail, especially in diluted solutions (Stevens and Elton 1971). Gelatinization of potato starch (Donovan 1979) and wheat starch (Wootton and Bamunuarachchi 1979) in concentrated systems was recently studied also. The thermal behavior of the protein part of a dough, ie, the gluten proteins, is still unknown, however. Gluten is defined as the part of a dough remaining when starch and other water-soluble components have been washed away with excess water. The water content of gluten is 60–70%. Dry gluten is composed of 75–80% protein, 5–15% starch, 5–10% lipids, and small quantities of mineral salts (Pyler 1973).

Differential scanning calorimetry (DSC) is a method well suited for the study of thermal denaturation processes (Hegg et al 1978). So far DSC has not been used much either in the study of the baking process or in the examination of dough constituents during a thermal treatment (Donovan 1977).

In this investigation, the thermal behavior of gluten is reported and an attempt is made to identify the different peaks in the DSC thermogram. The thermal transitions of gluten are also compared with those of a dough.

MATERIALS AND METHODS

Materials

Gluten, starch, and dough were prepared from the Swedish winter wheat STARKE II. This flour contained 9.6% protein on a

dry weight basis. Gluten was prepared using a semiautomatic gluten washer (Glutomatic, Falling Number, Stockholm, Sweden). The moisture content of the gluten was 63%, determined as described in AACC method 44-19. Starch was obtained by centrifugation of the washing water from the gluten washer and was dried in air at room temperature overnight. Doughs were prepared at 25°C from 10 g of flour and 5.5 ml of water and were mixed for 5 min at a speed of 62 rpm in a 10-g farinograph (Brabender, Duisburg, Germany) mixing bowl. Newly prepared doughs were used in all experiments.

Differential Scanning Calorimetry

Thermal transitions were measured by DSC performed on a

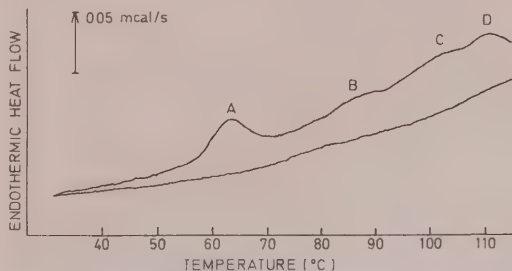


Fig. 1. The differential scanning calorimetry thermogram of gluten. Upper curve, gluten when heated at 10°C/min; lower curve, same gluten sample reheated after rapid cooling in the differential scanning calorimeter. Endothermic peaks A and D are probably the result of starch transitions, peaks B and C of protein denaturation.

Perkin-Elmer DSC-2. Aluminum sample pans (Perkin-Elmer No. 219-0062) were used. The amount of gluten, dough, or starch-water mixture was 10–15 mg in all experiments. A sealed sample pan enclosing a piece of silver of appropriate heat capacity was used as reference. The experiments were conducted at a scanning rate of 10°C/min. At lower scanning rates the peaks in gluten could not be observed because of a low signal to noise ratio.

Transition temperatures were defined as the intersection of the extrapolated lower temperature side and the extrapolated higher temperature side of the DSC peak. Calibration of the DSC and calculation of apparent transition enthalpies have been described earlier (Hegg et al 1978). All reported values are the means of three to six independent replicates.

RESULTS AND DISCUSSION

Figure 1 shows the DSC thermogram of gluten. Four endothermic peaks are observed when the temperature is linearly increased from 30° to 115°C. The corresponding transition temperatures and apparent transition enthalpies are given in Table I.

Donovan (1977) has reported that wheat starch gelatinization occurs near 65°C; the endotherm registered at 64.6°C (peak A) must therefore be caused by gelatinization of starch remaining in the gluten. The gelatinization process could be distinguished from protein denaturation by an estimation of the enthalpy of the two processes. The area of the gelatinization peak, ie, the enthalpy of the process, is dependent on the ratio of water to starch (Donovan 1979). Thus, to elucidate the influence of starch gelatinization on peak A, DSC thermograms of starch-water mixtures with the same water content as that of gluten were recorded, and the energy per milligram of starch was estimated. The energy of peak A in the gluten thermogram was calculated and compared with the energy per milligram of starch. A starch contamination of 7% in the gluten preparation corresponded to the observed area of peak A. Because this degree of starch contamination is conceivable, the conclusion is reasonable that peak A is due to gelatinization of starch in the gluten preparation.

A second endothermic peak has been observed on potato starch at higher temperatures at certain water contents (Donovan 1979). Endothermic changes also occur on wheat starch at higher temperatures (Eliasson 1980). Consequently, from these DSC-recordings at different water to starch ratios, peak D in the thermogram of gluten can reasonably be assigned to transitions in starch also.

In contrast to peaks A and D, which thus could be explained by starch transitions, peaks B and C are more difficult to account for. The lipid part of gluten could not give rise to these transitions, because the thermogram of separated wheat lipids gave no peaks. The lipids were prepared as described by Carlson et al (1978). Consequently, assignment of these peaks to the gluten proteins is tempting. In protein denaturation, however, large enthalpies usually are involved (Donovan et al 1975, Hegg¹). The apparent enthalpies corresponding to peaks B and C, given in Table I, are about 100 times less than that of an ordinary protein denaturation process. In spite of this, these peaks must be assigned to the proteins in gluten because separated gluten proteins gave enthalpies of the same magnitude. Further investigations on separated gluten proteins are in progress to elucidate whether peaks B and C could be assigned to any specific protein or class of proteins in gluten. Of the two peaks registered, only peak B (88.4°C) is of any interest in the baking process; peak C occurs at too high a temperature (101.4°C). The amount of ordered structure of the gluten proteins is uncertain (Kasarda et al 1976, Pyler 1973). The low endothermic heat flow registered in the thermal denaturation process could be interpreted either as lack of ordered structure or as stability of ordered structure towards heat. In any case, the gluten proteins seem to be marginally influenced by heat, and the term "thermal denaturation" might in this connection be misleading. However,

TABLE I.
Transition Temperatures and Transition Enthalpies of Gluten

Peak	Temperature (°C)	Apparent Transition Enthalpies (cal/g)
A	64.6 ± 1.6	0.35 ± 0.25
B	88.4 ± 1.8	0.06 ± 0.03
C	101.4 ± 1.6	0.03 ± 0.01
D	111.8 ± 0.8	0.08 ± 0.02

small changes crucial to the final baking result might occur during the heating process without a large endothermic heat flow.

To examine whether the four peaks in gluten could also be distinguished in a baking process, some experiments were performed on the complex system of dough. Two endothermic peaks of about equal size were registered. (Two peaks were observed earlier by Donovan (1977).) One peak was at 67.6 ± 0.4°C and the other at 90.6 ± 1.7°C. These two peaks and peaks A and B in gluten show rather good correlation, although the transition temperatures in dough are somewhat higher than those of gluten (Table I). Considering only the temperatures, the first peak in dough seems to be the result of starch gelatinization and the second to protein transitions. However, an estimation of the enthalpy for the second peak in dough reveals about 1 cal/g compared to 0.06 cal/g for peak B in gluten (Table I). Apparently, the thermal behavior of the gluten proteins cannot explain the second peak in the thermogram of dough. Further studies must be performed before any conclusions can be drawn about this second peak in the baking process, but DSC thermograms may possibly arise not only from denaturation of a single component but also from interactions between components.

In conclusion, thermal transitions of proteinous material appear to occur in gluten, but the enthalpies involved are very small. These small enthalpies do not support the idea of protein denaturation as an important factor in the baking process.

ACKNOWLEDGMENTS

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¹Unpublished results

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Some Effects of Starch Lipids on the Thermal and Rheological Properties of Wheat Starch

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Thermal and rheological properties of starches from two different wheat varieties were examined in relation to differences in surface distribution of lipids in the starch granules. The lipids present on the outside of the granules were extracted by isopropanol and the lipids in the amylose lipid complexes were extracted by water-saturated butanol. These amylose lipid complexes influence both the thermal properties of the starch, measured by differential scanning calorimetry, and the viscosity of the starch. The starch with the greatest amount of lipids extractable by water-saturated butanol began to gelatinize at a higher temperature and the gelatinization enthalpy was lower. Furthermore the viscosity began to increase at a higher temperature and the maximum viscosity was achieved at a higher temperature for this starch.

Einige Wirkungen von Stärkelipiden auf die thermischen und rheologischen Eigenschaften von Weizenstärke. Die thermischen und rheologischen Eigenschaften von Stärken von zwei verschiedenen Weizenarten wurden auf Unterschiede hinsichtlich der Oberflächenverteilung von Lipiden in den Stärkekörnern untersucht. Die Lipide an der Außenseite der Körner wurden mit Isopropanol und die Lipide in den Amylosekomplexen mit wassergesättigtem Butanol extrahiert. Diese Amyloselipide beeinflussen sowohl die thermischen Eigenschaften der Stärke, ermittelt mit Hilfe der Differential-Raster-Kalorimetrie, als auch ihre Viskosität. Die Stärke mit der größten Menge an mit wassergesättigtem Butanol extrahierbaren Lipiden begann bei einer höheren Temperatur zu gelatinieren, und die Enthalpie der Gelatinierung war niedriger. Außerdem fing die Viskosität bei höherer Temperatur an zu steigen, und das Maximum der Viskosität für diese Stärke wurde bei einer höheren Temperatur erreicht.

1 Introduction

In previous studies of physical properties of wheat lipids [1, 2] we observed certain differences in phase behaviour between aqueous systems of wheat flour lipids and lipids extracted from gluten. As the wheat flour was extracted without gelatinization of the starch it was in this connection of interest to examine the lipids obtained by the same type of extraction of starch. From work by Morrison et al. [3] it is known that considerable amounts of starch lipids can be obtained without gelatinization, and Acker has shown [cf. Ref. 4] that lipids incorporated in the first stages of the ripening consist mainly of fatty acids and monoglycerides, whereas lysolecithin dominates in the later stages. From their work it is obvious that water-saturated butanol is able to extract lipids from the outer part of the starch granules by replacement of the lipids in the helical amylose inclusion complexes. The present work concerns these lipids, and in order to separate them from lipids present on the outside of the granules, a first extraction

step by isopropanol was introduced. The protein content on the surface of the starch granules was determined and the proteins were also analysed using electrophoresis. Wheat of two different genetic origins, with good and poor baking performance, were compared.

From work by Lindqvist [5] it is known, that a necessary condition for cold gelatinization of starch is that amylose molecules leach out from the starch granules. Based on this proposed gelatinization mechanism it should be expected that lipid complexing of amylose will reduce the degree of swelling, and it has also been observed that the gelatinization can be completely inhibited in the presence of lipids in the outside water medium [6].

It might then be expected that differences in the surface distribution of lipids in the granules should influence the viscosity of the starch suspension. Thermal properties of the two starches, when heated at conditions similar to those of the baking process, were studied with differential scanning calorimetry (DSC).

2 Experimental

2.1 Materials

Starches from two different wheat varieties were used, *Amy* with good baking performance and *Maris Huntsman* with poor baking performance.

2.2 Methods

2.2.1 Preparation of starch

The starch was isolated from wheat grains by the steeping procedure described by Meredith et al. [7] except that the fractionation by successive sedimentation was excluded in this investigation. The starch was air dried. When the starch was isolated from flour, a Glutomatic separation device was used. About 200 ml distilled water was used for 10 g flour, and this starch suspension was centrifuged at 1900xg during 30 min. The starch was separated and washed once in 200 ml distilled water, and after centrifugation (the same conditions) the first grade (primary) fraction and the second grade (secondary) fraction were separated according to Ref. [8]. The primary fraction was used for viscosity measurements.

2.2.2 Lipid extraction

Extraction with isopropanol was performed using 10 ml of solvent per g starch during one hour in a shaking device. The solvent was separated by centrifugation and the starch was then washed once with isopropanol. The amount of lipids was determined by weighing after evaporation. The isopropanol-extracted starch was extracted by butanol-water 80:20 (v/v) using the same procedure as in the case of isopropanol. The butanol-water mixture was dried over P_2O_5 to constant weight after evaporation of the solvent. Thin layer chromatography (TLC) was also used to characterize the lipids as described earlier [1].

2.2.3 Protein determination

The amount of nitrogen was determined by conventional Kjeldahl analysis and it was converted to protein by the factor 5.7. The proteins were also analysed with sodiumdodecylsulphate (SDS)-electrophoresis [9]. The proteins outside the starch granules were extracted by a solution of mercaptoethanol and SDS.

2.2.4 Viscosity measurements

The viscosity of 6% (w/w) starch suspensions in water was measured using a Rotovisko RV3. The measurements were started at 70°C in order to avoid starch sedimentation during the measurements. The starch suspension was tempered at 70°C for 5 min before it was added and the measurement started. The temperature was then increased 1.5°C/min up to 91–92°C. After about 10 min at this temperature, cooling was started (1.5°C/min).

2.2.5 Differential scanning calorimetry

The thermal properties of the starches before and after lipid extraction were studied with differential scanning calorimetry. The measurements were carried out on a Perkin-Elmer DSC-2, at a scanning rate of 10°C/min. The water contents in the studied starch-water mixtures were in the region 25–55% (w/w). 1–15 mg of the starch-water mixture was used for the measurements. An empty pan was used as reference. The transition temperatures are defined in Figure 5. The

calibration of the DSC and the calculation of the enthalpies are described earlier [10]. The reported values are the means of three independent triplicates.

3 Results and Discussion

The amounts of lipids extracted in isopropanol and in butanol-water are given in Table 1. Examples of the TLC-patterns are given in Figure 1. It should be expected that isopropanol only can extract lipids outside the starch granule. The lipids, which dominate in the isopropanol extract (Fig. 1), form bilayers in water, which is a characteristic feature of membrane lipids. If the starch granule is assumed to be covered by a lipid bilayer (thickness about 50 Å) the proportion of lipids for a hypothetical A-granule (assumed to be spherical, with a diameter of 30 µ) is about 0.1% (w/w). As can be seen in Table 1 the amount of lipids extracted by isopropanol is somewhat smaller.

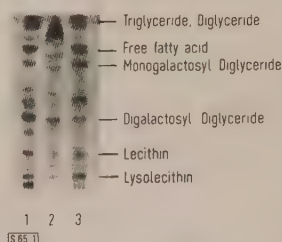


Figure 1. TLC-patterns of lipids extracted from the wheat variety *Amy*. 1 = Total lipids extracted from flour by water-saturated butanol. 2 = Lipids extracted from starch by isopropanol. 3 = Lipids extracted from starch by water-saturated butanol.

Table 1.
Amounts of Extracted Lipids.

Starch	Extraction by isopropanol (mg/g)	Extraction by butanol-water (mg/g)
<i>Amy</i>	0.9	1.7
<i>Maris Huntsman</i>	0.2	1.1

The amounts of proteins estimated from the starch fractions are given in Table 2, and are in general agreement with values reported earlier [cf. Ref. 8]. Extraction of the lipids did not significantly influence the amounts of protein obtained, considering that contaminating lipids also contribute to the nitrogen value [11].

Table 2.
Amount of Protein on the Surface of Starch Granules.

Starch	N (mg/g)	Protein (mg/g)
<i>Amy</i>	0.83	4.7
<i>Maris Huntsman</i>	0.26	1.5

The electrophoretic results (Fig.2) show that gliadins are present on the starch granules. The molecular weights of the proteins extracted from the starch granules are in agreement with the molecular weights of wheat gluten subunits, determined by SDS-electrophoresis [12].

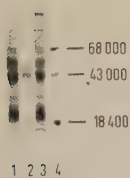


Figure 2. Electrophoretic patterns of proteins extracted from starch prepared from the wheat variety *Amy*. 1 = Proteins extracted from starch before removal of lipids. 2 = Gliadins from *Amy*. 3 = Proteins extracted from starch after removal of lipids. 4 = Molecular weight markers: bovine serum albumin, ovalbumin and β -lactoglobulin.

An important property of starch from a surface chemical point of view is that it is wetted and spontaneously dispersible in water as well as in oil. This means that the surface is able to either exhibit hydrophobic or hydrophilic groups. A lipid bilayer structure can easily adopt to such changes in surface properties.

In a work by Cauvain et al. [13] "non-starch" material outside the starch granules was removed by repeated washings by toluene and water. In this way the amount of protein was reduced from 0.23% to 0.19% and 'fat' as determined after acid hydrolysis from 0.50% to 0.48%. In comparison with the extraction procedure used in the present work relatively small amounts of protein and lipids were obviously removed. Still they observed clear differences in pasting behaviour and baking performance, which were attributed to surface properties.

From Table 1 it can be concluded that the starch granules from *Amy* are surrounded by a thicker lipid layer than the starch granules from *Maris Huntsman* provided that the distributions of granule sizes in the starches are equal [see Ref. 7]. Furthermore the difference in butanol extractability indicates that more amylose lipid complexing occurs in *Amy* than in *Maris Huntsman*. Both these conditions could be expected to influence the gelatinization of the starch. The outer lipid layer can act partly as a diffusion barrier and partly as a lipid depot for amylose complexing, whereas the amylose-lipid complex within the starch granules might be regarded as an immobilization of the amylose monomers. A thick protein coating could also delay the water transport to the surface of the starch granule, and also prevent the amylose to leach out from the starch granules.

The viscosity versus temperature is shown in Figure 3 for *Amy* and *Maris Huntsman* before and after the lipids were extracted. The viscosity begins to increase at a higher temperature with *Amy* (the starch with the greatest amount of extractable lipids), and the peak viscosity is lowest for this starch. When the lipids have been removed, the curves are shifted so that the viscosity begins to increase at a lower temperature, and the maximum viscosity is also shifted to a lower temperature. The peak viscosity shows an increase for *Amy*. These features are in agreement with the lipid-amylose interactions discussed above. The same effect of delipidization on the viscosity of starch has been reported earlier (cf. Ref. [14]).

The starch samples used in this investigation were prepared by a 'mild' method in order to avoid starch damage, both due to mechanical treatment and to amylose activity. In Figure 4 the viscosity curve for delipidized starch prepared from the flour is compared with the viscosity curve for delipidized undamaged starch, both starch samples prepared from *Maris*

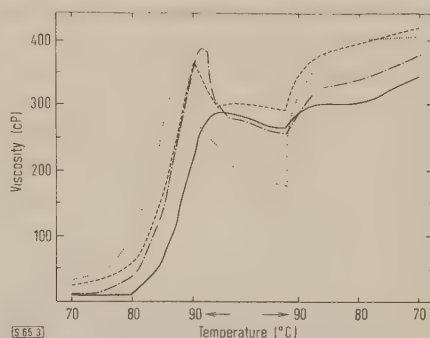


Figure 3. Viscosity curves for starches from *Amy* and *Maris Huntsman*. — *Amy*, --- *Amy* after removal of lipids, ... *Maris Huntsman*, -.- *Maris Huntsman* after removal of lipids.

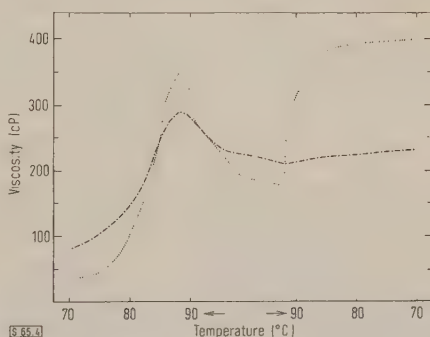


Figure 4. Viscosity curves for delipidized starches from *Maris Huntsman*. ... Starch prepared from grains, --- starch prepared from flour.

Huntsman. The poor viscosity characteristic of flour from *Maris Huntsman* is probably due to amylose activity. Starch from *Maris Huntsman* is probably more susceptible to amylose breakdown since amylose in an amylose lipid complex is protected against amylose attack to a certain degree [15], and there is less complex present in this wheat variety than in *Amy*.

The viscosity measurements give information about the rheological behaviour of the starch gel when it is heated during mechanical stirring. The measurements start at a temperature above the gelatinization temperature and the viscosity is measured in a very dilute starch suspension [94% (w/w) water]. One important feature is the adhesion between the swollen starch granules. In the DSC-measurements, on the other hand, the changes inside the individual granules are measured. The measurements start at a temperature below the gelatinization temperature, and the water content in the starch sample may be varied over a broad range. It is a wellknown fact that not all the starch granules in a starch sample have the same gelatinization temperature, so that the starch gelatinizes over a broad temperature interval. Thus, the peaks observed on the DSC-thermograms are the sums of the energies involved in the thermal processes occurring in the individual starch granules.

DSC was used to study the influence of the amylose lipid inclusion complex on the thermal properties of wheat starch. Starch from *Maris Huntsman*, before and after removal of

lipids, and *Amy*, after removal of lipids, were compared. When wheat starch is heated at the scanning rate of 10 deg/min three endothermic processes occur at certain starch to water ratios [16]. This is illustrated in Figure 5, where also the nomenclature of the transition temperatures is explained. From Table 3 it can be seen that the gelatinization starts at lower temperatures for the starch with the smallest amount of extractable lipids (*Maris Huntsman*). The tendency is the same in the viscosity measurements (Fig. 3), where the viscosity begins to increase at a lower temperature for *Maris Huntsman*. The peak temperature, T_p , seems not to vary with the amount of extractable lipids. The temperature of the second process, T_{II} , is lower for *Maris Huntsman* than for *Amy* [except at a water content of 35% (w/w)]. The difference is greatest at 40% (w/w) water and becomes smaller when the water content is increased. The temperature of the third process, T_{III} is the same for both starches.

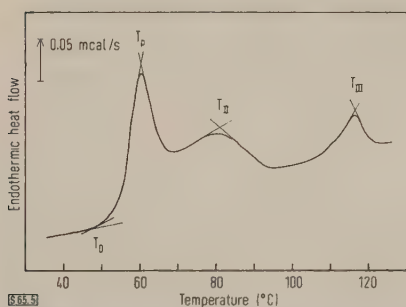


Figure 5. DSC-thermogram for starch from *Maris Huntsman*. The water content is 50% (w/w) and the amount of starch in the sample is 5.070 mg. T_0 , T_p (gelatinization peak), T_{II} and T_{III} are defined as indicated in the figure.

The enthalpies of the first and second processes are shown in Figures 6 and 7. At the water contents studied, the enthalpy for the gelatinization was somewhat greater for *Maris Huntsman* than for *Amy* (Fig. 6). This was also the case for the second process at water contents below 45% (w/w) (Fig. 7). If the gelatinization enthalpy at a certain water content is interpreted as a measure of the degree of gelatinization, the difference between *Amy* and *Maris Huntsman* may be explained by the differences in extractable lipids. A smaller amount of amylose forms the lipid complex in the starch from *Maris Huntsman*, and thus the amylose can easier leave the granule and the starch gelatinizes to a greater extent than the starch from *Amy*.

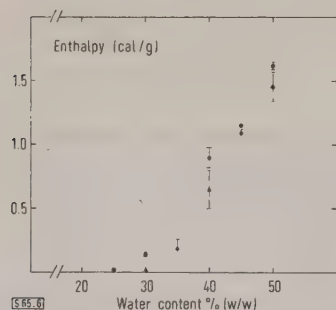


Figure 6. The gelatinization enthalpy. Δ *Amy*, $\bullet$ *Maris Huntsman*.

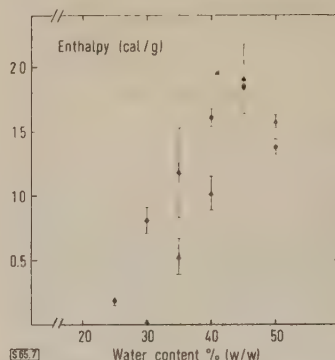


Figure 7. The enthalpy of the second process. Δ *Amy*, $\bullet$ *Maris Huntsman*.

The enthalpies of the first and second processes, when the lipids are extracted, are shown in Figures 8 and 9. The butanol extracts the lipids from the outer part of the starch granules by replacement of the lipids in the helical amylose inclusion complex. During the extraction of the lipids, butanol is present in excess, and the lipids in the complexes are replaced by butanol.

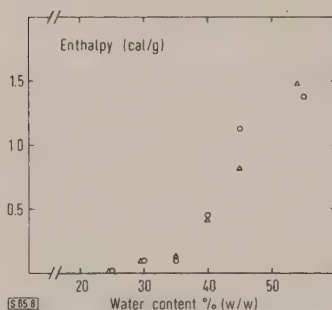


Figure 8. The gelatinization enthalpy for the starches when the lipids are removed. Δ *Amy*, $\circ$ *Maris Huntsman*.

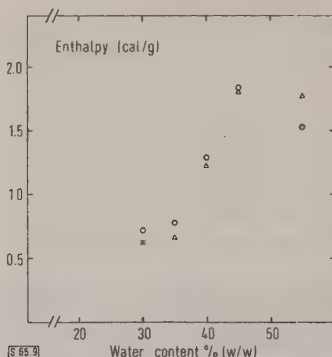


Figure 9. The enthalpy of the second process for starches when the lipids are removed. Δ *Amy*, $\circ$ *Maris Huntsman*.

Also new amylose butanol complexes may be formed during the extraction. The effect of removal of the lipids on the temperatures in Table 3, may be explained by butanol remaining in the starch. Especially at a water content of 35% (w/w) the effect is obvious on T_0 . The influence is smaller on T_p and T_{II} , whereas T_{III} is influenced neither of wheat variety nor of lipid extraction. At this high temperature the contribution from the surface zone of the granules on the thermal properties is probably hidden, as the third process is a result of changes throughout the whole granule.

Table 3.
Endotherm Temperatures for Starches from *Amy* and *Maris Huntsman* Before and After Removal of Lipids. T_0 , T_p , T_{II} and T_{III} are defined as in Figure 5.

Starch	Water content % (w/w)	T_0 (°C)	T_p (°C)	T_{II} (°C)	T_{III} (°C)
Before removal of lipids					
<i>Amy</i>	35	50	60	106	139
<i>Maris Huntsman</i>	35	49	58	108	140
<i>Amy</i>	40	51	60	98	128
<i>Maris Huntsman</i>	40	49	61	89	—
<i>Amy</i>	45	50	62	88	121
<i>Maris Huntsman</i>	45	46	62	87	122
After removal of lipids					
<i>Amy</i>	35	52	60	105	140
<i>Maris Huntsman</i>	35	53	60	107	—
<i>Amy</i>	40	48	61	98	129
<i>Maris Huntsman</i>	40	50	60	94	128
<i>Amy</i>	45	50	63	91	122
<i>Maris Huntsman</i>	45	44	62	88	122

The effect of the removal of lipids on the gelatinization enthalpy is shown in Figure 8. The enthalpy at a certain water content is lowered when the lipids are removed, and the differences between the starches are smaller. The trend is the same for the second process (Fig. 9). This is in agreement with the discussion above, i. e. that butanol replaces the lipids in the amylose complex and even forms new complexes. About the same amount of butanol amylose complex is present after the extraction, independent of the amount of lipids in the original starch. At water contents lower than 35% (w/w) the picture is somewhat more complicated, and for the delipidized starches it is impossible to extrapolate the gelatinization enthalpy to zero.

The DSC-thermograms for starch from *Amy*, with and without lipids, are compared with starch from *Maris Huntsman*, at a water content of 30% (w/w) in Figure 10. Starch from *Amy* does not gelatinize at this water content, but after extraction by butanol it does. *Maris Huntsman* gelatinizes at a water content of 30% (w/w) but not at 25% (w/w).

We have also examined other wheat varieties and observed similar relations between extracted lipids and technical properties as in the case of *Amy* and *Maris Huntsman*

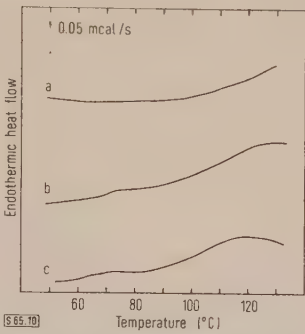


Figure 10. DSC-thermograms for starch-water-mixtures, where the water content is 30% (w/w). a = Starch from *Amy*, 2.138 mg starch in the sample. b = delipidized starch from *Amy*, 2.475 g starch in the sample. c = starch from *Maris Huntsman*, 3.198 mg starch in the sample.

discussed above. Differences in thermal and rheological properties of starch from different wheat varieties are thus clearly related to differences in surface distribution of lipids in the starch granule.

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IV

ON THE POSSIBILITY OF MODIFYING THE GELATINIZATION
PROPERTIES OF STARCH BY LIPID SURFACE COATING

by

A.-C. Eliasson, K. Larsson and Y. Mieziš

On the Possibility of Modifying the Gelatinization Properties of Starch by Lipid Surface Coating

By A.-C. Eliasson, K. Larsson
and Y. Mieziš, Lund

The gelatinization properties of potato starch were modified by coating the surface of the starch granules with lipids by an aqueous dispersion of monoglyceride or an ethanol monoglyceride solution. The volumes of gels were smaller than those of the untreated starch. In the polarizing microscope it was observed that decreased disruption of starch granules in connection with gelatinization occurs with increasing amount of lipids present on the surface. The gelatinization enthalpy and the gelatinization temperature at a low water content measured by DSC (Differential Scanning Calorimetry) were not influenced by the lipid coating. A third endothermic transition, however, was observed for the lipid-coated starch. Freeze-drying, used in the preparation of the lipid-coated starch, was found to lower the gelatinization enthalpy as well as the gelatinization temperature.

Über die Möglichkeit zur Modifizierung der Verkleisterungseigenschaften von Stärke durch Oberflächenbeschichtung mit Lipiden. Die Verkleisterungseigenschaften von Kartoffelstärke wurden durch Beschichtung der Oberfläche der Stärkekörner mit Lipiden mittels einer wäßrigen Dispersion von Monoglycerid oder einer ethanolischen Monoglyceridlösung modifiziert. Die Gelvolumina waren kleiner als die der unbehandelten Stärke. Im Polarisationsmikroskop wurde beobachtet, daß das mit der Verkleinerung verbundene Zerreißen der Stärkekörner mit zunehmender Lipidmenge auf der Oberfläche vermindert wurde. Die Verkleinerungsenthalpie sowie die Verkleinerungstemperatur bei niedrigem Wassergehalt – gemessen durch DSC (Differential-Raster-Kalorimetrie) – wurden nicht beeinflusst. Ein dritter thermischer Phasenübergang wurde jedoch beobachtet. Es wurde gefunden, daß die für die Darstellung der lipidbeschichteten Stärke angewendete Gefriertrocknung sowohl die Verkleisterungsenthalpie als auch die Verkleisterungstemperatur erniedrigte.

1 Introduction

In a recent study differences in thermal and rheological properties between starches from different wheat varieties were related to differences in the surface distribution patterns of lipids in the starch granules [1]. The native occurrence of a lysolecithin-amylose inclusion complex in wheat, where the hydrocarbon chain is surrounded by an amylose helix (the V-amylose form), has been conclusively demonstrated, particularly from work by Acker [2]. Such complexing of amylose by lipids should be expected to influence the thermal and rheological properties of the wheat starch, by reducing the transport of amylose molecules from the granules to the water, and thus influence the thermal gelatinization in a similar way as the amylose-iodine complex influences cold gelatinization [3]. The thermal gelatinization of potato starch, which is almost free from lipids, can be inhibited, provided that enough of lipid monomers are distributed to the surface of the granule to complex with leaching amylose [4]. These findings indicate that there are possibilities of modifying the gelatinization properties of starch by adding a surface coating of lipids. Such studies are reported below.

Based on earlier results [5], a dispersion of a monoglyceride of sunflower oil was used in order to obtain a surface coating by a mono-acyl lipid. This compound was selected as it is used as a food additive and has a liquid-type of hydrocarbon chain structure, forming liquid-crystalline phases with water at temperatures between room temperature and the starch gelatinization temperature. A complication, however, is that it forms a cubic phase with water [5], which cannot be dispersed unless a micellar type of surfactant is present. Small amounts of a bile salt, sodium cholate (about 5% (w/w) of the

total amount of lipids) were used to form a homogeneous dispersion of the cubic phase in water. This dispersion was found to be effective in order to form a surface film on starch granules after freeze-drying. As an alternative we also used sodium caseinate, a food grade substance, to give the dispersion.

In the preparation of the lipid-coated starch samples, freeze-drying was used. There are several reports which show that freeze-drying gives rather strong effects on the physical properties of starch. The crystallinity of wheat starch, for example, is lower for freeze-dried starch than for air-dried starch [6]. Freeze-drying of flour increases flour water absorption [7], and the result of test baking from reconstituted flours is improved when air-dried starch is used instead of freeze-dried starch [8]. Thus it was necessary to study the effects of freeze-drying some more in detail to separate these effects from the effect of added lipids on the properties of potato starch.

In order to study the thermal properties of the starch samples, gel formation was followed visually, in the polarizing microscope, and by Differential Scanning Calorimetry (DSC).

2 Materials and Methods

2.1 Materials

Potato starch was obtained from Svenska Stärkelseproducenters Förening, Karlshamn (Sweden).

The technical monoglyceride used was an industrially distilled sunflower oil monoglyceride kindly supplied by A/S Grindstedvaerket, Brabrand (Denmark).

2.2 Methods

The water content of the starch was calculated from weight loss after heating at 135 °C for two hours. The measurements on the freeze-dried starches were not carried out until the starches had equilibrated in air to a water content of about 14% (w/w). The content of damaged starch was estimated by staining the starch in aqueous Congo-red (0.35%) and counting of the number of stained granules [9].

The lipid coated potato starches were prepared in the following way: 0.5 g of the monoglyceride was allowed to swell in 9.5 g of water to give the cubic phase. 0.025 g sodium cholate or sodium caseinate was added and this mixture was sonicated for two min to give a homogeneous dispersion. 0.5 g, 1 g and 2 g of this dispersion were mixed with water to 4 g each, potato starch was added and the suspensions were freeze-dried. The lipid contents of the freeze-dried lipid-starch samples were 0.7, 1.4 and 2.8% (w/w), respectively.

To compare gel volumes, 1% (w/w) suspensions of starch in water were heated in test tubes from room temperature to 50 °C in 5 min, then the temperature was increased to 70 °C at a heating rate of 1 °C/min.

Samples were examined in the polarizing microscope during the gelatinization. The starch sample with excess of water was placed on a microscope slide and heated at a rate of 1 °C/min using a Mettler FP5 heating equipment. At 60 °C, 65 °C and 70 °C, respectively, the heating was interrupted and photographs were taken.

The differential scanning calorimeter used was a Perkin Elmer DSC-2. Aluminium sample pans (Perkin Elmer No. 219-0062) were used. As reference a sealed sample pan was used, which enclosed a piece of silver of appropriate heat capacity. The amount of starch-water suspension was 5–15 mg and the water-to-starch ratios in the samples were in the range 1:1–5:1. The experiments were carried out at a scanning rate of 10 °C/min, except when the influence of the scanning rate was studied, when scanning rates of 5 °C/min and 1.25 °C/min were used.

The calibration of the instrument has been described elsewhere [10]. The transition temperatures given below are defined as the intersection of the extrapolated lower temperature side and the extrapolated higher temperature side of the DSC-peak. The enthalpies given, calculated from the areas of the endotherms, are the mean of 2–3 independent measurements. The transition temperatures (in Tables 1–4) were estimated to be accurate within ± 1 °C, and the enthalpies within ± 5 %.

In order to study the influence of freeze-drying on the gelatinization temperature for potato starch, a suspension of potato starch in water without added lipids was freeze-dried in the same way as the starch-lipid preparations. Furthermore in order to study the effects of freezing and drying separately, one sample of potato starch was frozen with its original water content, another sample was frozen with excess of water (water-to-starch ratio was 5:1), and a third sample was dried at 135 °C for 3 h. Prior to the calorimetry experiments the frozen and thawed dry starch and the oven-dried starch were mixed with water to give a water-to-starch ratio of 5:1. The starch frozen with this water content was only thawed.

3 Results and Discussion

3.1 Gel volumes

The volume of the potato starch gel is reduced when lipids are present on the starch granule surface (Fig. 1). The decrease in gel volume is depending on the amount of added lipid. From

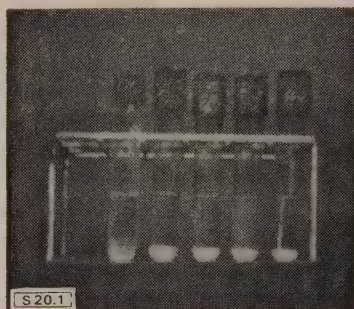


Figure 1. The volumes of gels formed when 1% (w/w) aqueous suspensions of different starches are heated at a heating rate of 1 °C/min to 70 °C. The test tubes contain from left to right freeze-dried potato starch without added lipids, starch of the same type with 0.7% (w/w) lipids, starch with 1.4% (w/w) lipids, starch with 2.8% (w/w) lipids, and for comparison wheat starch.

Figure 1 it can be seen that the potato starch with 2.8% (w/w) lipids gives a gel volume which is about the same as the volume of a wheat starch gel formed during the same conditions. It is also evident from Figure 2 that the gel forming properties of the potato starch are changed by freeze-drying, since the freeze-dried starch gives a distinctly smaller gel volume compared to the original potato starch.



Figure 2. The volume of the gel formed by freeze-dried potato starch (right) compared to the original potato starch (left) when 1% (w/w) aqueous suspensions are heated at a heating rate of 1 °C/min to 70 °C.

3.2 Microscopy

The appearance of the starch granules during the gelatinization was studied in microscope. Freeze-dried potato starch without added lipids was compared with potato starch with 2.8% (w/w) lipids when the starches are heated to 60 °C (Fig. 3b and 3c), 65 °C (Fig. 3d and 3e) and 70 °C (Fig. 3f and 3g). The original potato starch heated to 60 °C is shown in Figure 3a for comparison.

The texture of the starch granules when heated to 60 °C (Fig. 3a, 3b and 3c) shows that the freeze-dried potato starch (either with or without added lipids) gelatinizes to a greater extent at this temperature than the original potato starch. The starch granules without lipids are disrupted at lower temperatures than the lipid-coated granules. At 70 °C it is impossible to distinguish any individual starch granules without added lipids (3f), whereas it is still possible to distinguish individual

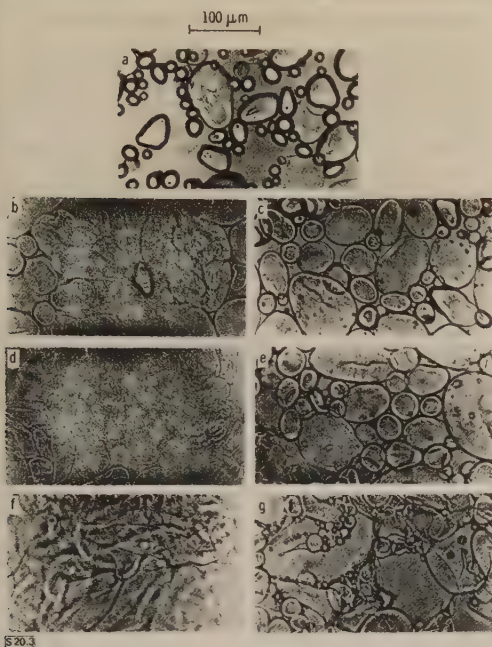


Figure 3. The appearance of the starch in the microscope; a = potato starch heated to 60°C; b, d and f = freeze-dried potato starch heated to 60°C (b), 65°C (d) and 70°C (f); c, e and g = freeze-dried potato starch with 2.8% (w/w) lipids heated to 60°C (c), 65°C (e) and 70°C (g).

lipid-coated starch granules at 70°C (Fig. 3g), although they are strongly swollen.

3.3 Thermal transitions of freeze-dried starch

DSC-thermograms for the original potato starch and the freeze-dried potato starch are given in Figure 4. The most striking difference between these thermograms is the shift in gelatinization temperature. Freeze-drying lowers the gelatinization temperature from 66°C to 60°C. The gelatinization temperatures for potato starch frozen and dried in different ways are given in Table 1. The drying of the starch at 135°C does not cause gelatinization, since the water content is too low [11]. From Table 1 it is evident that the decrease in gelatinization temperature is due to the drying process and not to the freezing of the starch.

Table 1. Gelatinization Temperatures for Potato Starch Measured by DSC after Different Freezing and Drying Methods. The Gelatinization Temperatures are Measured at a Scanning Rate of 10°C/min and the water-to-starch ratio in the samples is 5:1.

Starch	Gelatinization temperature (°C)
Potato starch	66
Freeze-dried potato starch	60
Potato starch frozen with water (water-to-starch ratio was 5:1)	66
Potato starch frozen without extra water	66
Potato starch dried at 135°C for 3 h	61

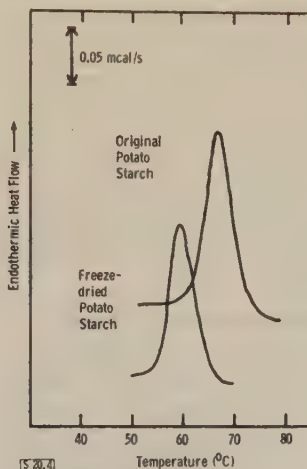


Figure 4. Thermograms for the original potato starch and the freeze-dried potato starch at a scanning rate of 10°C/min. The amount of starch is 2.21 mg and 1.37 mg, respectively, and the water-to-starch ratio is 5:1.

The gelatinization enthalpies for the original potato starch and the freeze-dried potato starch were measured at a few water-to-starch ratios and the results are given in Table 2. The enthalpies for the original potato starch are in agreement with earlier reported values [11, 12]. The enthalpies for the freeze-dried potato starch are lower over the whole water content range investigated, also observed by Ahmed and Lelièvre [6]. At certain water contents, two endothermic transitions are observed for potato starch [11]. Two endothermic transitions are also observed for the freeze-dried potato starch and these transitions coexist at the same water-to-starch ratios for the freeze-dried potato starch as for the original potato starch. Thus, at a water-to-starch ratio of 2:1, the gelatinization endotherm is the only endotherm observed both for the original potato starch and for the freeze-dried starch.

Table 2. Influence of Water Content on the Gelatinization Enthalpy for the Original Potato Starch and the Freeze-dried Potato Starch at a Scanning Rate of 10°C/min.

Water-to-starch ratio	ΔH (cal/g starch)	
	Original starch	Freeze-dried starch
1:1	2.3	0.8
1.2:1	3.3	2.3
2:1	5.2	3.9

On the basis of a DSC-study Stevens and Elton [12] reported that damaged starch granules caused a lowering of the gelatinization enthalpy for wheat starch. The possibility of an increase in the number of damaged starch granules due to freeze-drying was therefore investigated. The number of granules stained by Congo-red was counted, as well as the number of granules which showed no birefringence in the polarizing microscope. The share of damaged starch granules was about 2% both for the original potato starch and the freeze-dried potato starch, and no increase in the number of

damaged starch granules could be detected in the freeze-dried starch. When the starch granules had been suspended in the aqueous solution of Congo-red over night before examination in the microscope, the freeze-dried starch granules were stained slightly orange-red, whereas the original potato starch granules were uninfluenced. The faintly stained granules were still birefringent. The freeze-drying thus seems to alter the structure of the starch granule in a way which allows more water to penetrate the starch granule already at room temperature. The lower gelatinization enthalpy for the freeze-dried potato starch should then be due to the fact that a lesser amount of water penetrates the starch granule when the temperature is increased.

3.4 Thermal transitions of lipid-coated potato starch

Gelatinization enthalpies and temperatures for the lipid-coated potato starch were measured by DSC at a water-to-starch ratio of 2:1. The measured enthalpies and temperatures are given in Table 3. Considering the uncertainty in the measurements of the enthalpy, the values can be regarded as independent of the amount of added lipids. Also the gelatinization temperatures seem to be independent of the presence of lipid-coating on the starch granule surface (Table 3) within the experimental error.

Table 3.
Gelatinization Enthalpy and Gelatinization Temperature for Potato Starch with Different Amounts of Added Lipids. The Measurements Are Carried out a Scanning Rate of 10°C/min and the Water-to-Starch Ratio is 2:1 in All Samples.

Amount of added lipids (weight-%)	ΔH (cal/g starch)	Gelatinization temperature (°C)
No lipids added	3.9	59
0.7	3.9	59
1.4	3.8	58
2.8	3.7	59

A critical factor for the effects of lipids on starch gelatinization is the lipid monomer concentration in comparison with the velocity of thermal gelatinization [4]. The heating rate in the DSC-experiments shown in Table 3 was 10°C/min, compared with 1°C/min when the gels in Figure 1 were formed. The influence of heating rate on the gelatinization enthalpy for the potato starch with 2.8% (w/w) lipids was therefore investigated. The results are given in Table 4. Comparison of the enthalpies for this starch with corresponding enthalpies for the freeze-dried starch without added lipids shows that the lowering of the scanning rate influences the two starches to the same extent.

Table 4.
Influence of the Scanning Rate on the Gelatinization Enthalpies Measured by DSC. Water-to-Starch Ratio is 2:1.

Scanning rate (°C/min)	ΔH (cal/g starch)	
	No added lipids	2.8% (w/w) lipids
10	3.9	3.7
5	3.7	3.6
1.25	1.5	1.4

3.5 Thermal transitions at high temperatures

DSC-thermograms are given in Figure 5 for starch without added lipids and with 0.7 and 2.8% (w/w) lipids, respectively. The potato starch without added lipids shows no endothermic transition in the high temperature region. The two potato starches with added lipids both show an endothermic transition near 100°C. The enthalpies of the endotherms were calculated and in the case of starch with 0.7% (w/w) lipids the enthalpy is 0.2 cal/g starch, and for the starch with 2.8% (w/w) lipids the enthalpy is 0.5 cal/g starch. Three endothermic transitions are observed for wheat starch at certain water contents [10, 13]. However, in potato starch only two endothermic transitions have been observed [11]. The third endothermic transition, observed at high temperatures for wheat starch, was recently assigned to disordering of amylose-lipid complex by Kugimiya et al. [14]. The presence of a high-temperature endotherm on the DSC-thermogram for lipid-coated starch thus indicates that amylose-lipid complexes are formed.

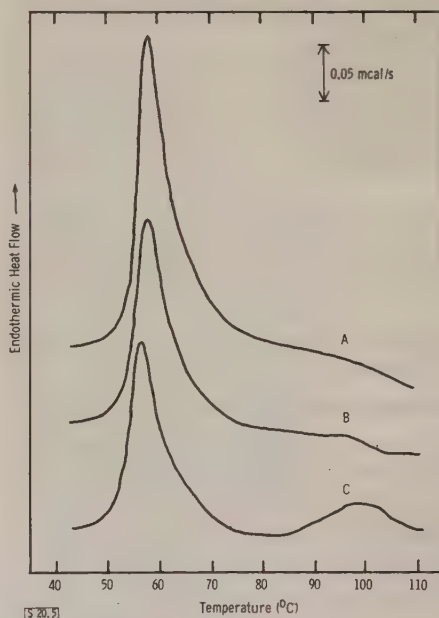


Figure 5. Thermograms at a scanning rate of 10°C/min for A = freeze-dried potato starch without added lipids, 3.46 mg starch in the sample pan; B = potato starch with 0.7% (w/w) lipids, 2.55 mg starch; C = potato starch with 2.8% (w/w) lipids, 2.28 mg starch. The water-to-starch ratio is 2:1 in all samples.

3.6 Gel modifications

A simple method to obtain a lipid coating on starch granules was also examined. Potato starch granules were suspended in an ethanol solution of monolaurin. After evaporation of the ethanol (in vacuum at room temperature), the starch lipid weight ratio was 98:2. Gelatinization studies showed a strong reduction in gel volume (about the same as the sample with 2.8% (w/w) lipids in Fig. 1), and observations in the polarizing microscope showed the presence of crystalline

regions contrary to the control (no lipid coating) which showed no birefringence.

The coating of starch by lipids provides a possibility to modify the physical properties of a starch gel, which might be of technical interest.

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V

RHEOLOGICAL PROPERTIES OF CONCENTRATED
WHEAT STARCH GELS

by

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Rheological Properties of Concentrated Wheat Starch Gels

By A.-C. Eliasson and L. Bohlin,
Lund (Sweden)

The rheological properties of concentrated wheat starch gels were measured with shear stress relaxation experiments in cone-plate geometry. The water content of the investigated gels were 80, 60, 50 and 30% (w/w), respectively, and the relaxation modulus (G) and half relaxation time ($T_{1/2}$) were studied after heating to different temperatures. For the gels with 50% and 60% (w/w) of water, a maximum value of G was obtained when the temperature interval for gelatinization was passed. When these gels were heated to higher temperatures, G decreased, and $T_{1/2}$ increased, whereas gels with 30 and 80% (w/w) of water behaved differently. The influence of particle size distribution on the rheological properties of concentrated starch gels, was also investigated. It was found that G and $T_{1/2}$ increased with a greater fraction of small starch granules.

Rheologische Eigenschaften von konzentrierten Weizenstärkegelen. Die rheologischen Eigenschaften von konzentrierten Weizenstärkegelen wurden durch Scherkraftentspannungsversuche mit Hilfe des Kegelplattenverfahrens gemessen. Die Wassergehalte der untersuchten Gele betrugen 80, 60, 50 und 30% (w/w), und der Entspannungsmodul (G) sowie die Entspannungs-Halbwertszeit ($T_{1/2}$) wurden nach Erhitzung auf verschiedene Temperaturen untersucht. Für die Gele mit 50 und 60% (w/w) Wasser wurde ein maximaler G -Wert erhalten, wenn das Temperaturintervall der Verkleisterung überschritten war. Wenn diese Gele auf höhere Temperaturen erhitzt wurden, verringerte sich G und erhöhte sich $T_{1/2}$, wogegen sich Gele mit 30 und 80% (w/w) Wasser unterschiedlich verhielten. Der Einfluß der Teilchengrößenverteilung auf die rheologischen Eigenschaften konzentrierter Stärkegele wurde ebenfalls untersucht. Es wurde gefunden, daß G und $T_{1/2}$ bei größerem Anteil kleiner Stärkekörner zunahm.

1 Introduction

When wheat starch is heated in the presence of limited amounts of water, three thermal transitions are observed by calorimetric measurements [1, 2]. At the lowest temperature (about 61 °C) an endotherm due to gelatinization is observed. A second endotherm at higher temperature may be observed when the water content is less than that necessary for complete gelatinization. The third endotherm is observed at temperatures above 100 °C, and this transition has been interpreted as a phase transition of the amylose-lipid complex [3]. In many food systems, e.g. in bread, limited amount of water is available for the starch, and the second thermal transition will then occur during heating, provided that the temperature is high enough. It would thus be of interest to investigate how the rheological properties of concentrated starch gels are related to the different thermal transitions. The present paper reports measurements of rheological properties of concentrated wheat starch gels with shear stress relaxation experiments in cone-plate geometry. Stress relaxation was measured on wheat starch gels containing different amounts of water after the starch-water mixtures had been heated to different temperatures.

The starch granules in wheat have a bimodal size distribution. The small granules are mainly spherical and have a diameter of about 2–8 µm, whereas the large granules are lens-shaped and have a diameter of about 25–35 µm [4]. It may be expected that the granule size influences the rheological properties of a starch gel due to different packing. It has been observed that the size of starch granules affects the rheological properties of composite doughs [5]. In this investigation the rheological properties of two starch preparations from the same wheat variety with different granule size distributions were compared.

2 Materials and Methods

2.1 Materials

The starch used was prepared from the Swedish spring wheat Amy. The water used was distilled and deionized.

2.2 Methods

2.2.1 Preparation of Starch

The starch was isolated from wheat grains by the steeping procedure described by Meredith et al. [6]. The primary starch, or A-starch, obtained after centrifugation was used. This starch was called "preparation I". In one experiment the residues of the grains were more fully washed and the yield of starch increased. This starch was called "preparation II". In this way, two preparations of starch with different particle size distributions were obtained. The starch preparations were air-dried. The water contents of the starches were determined by the AACC-method 44–15 [7], and these water contents were included in the total water content of the starch-water mixtures. In the following discussion the term "gel" will be used to denote a starch-water mixture which has been heated, either a gel really has been formed or not.

2.2.2 Particle Size Distribution

The particle size distribution of the two starch preparations was determined in a Coulter counter [8]. The size distributions were calculated as weight-% versus particle size diameter.

2.2.3 Differential Scanning Calorimetry

DSC-thermograms for the two starch preparations were obtained with a Perkin-Elmer DSC-2 at a scanning rate of 10 °C/min. About 10 mg of the starch-water mixture was transferred to a weighed aluminium sample pan (Perkin-Elmer no. 219-0062), which was sealed and reweighed. An empty pan was used as reference.

2.2.4 Heating of the Starch-Water Mixtures

Two grams of starch and water, to give a water content of 30, 50, 60 or 80% (w/w) were mixed in a test tube. The sealed test tube, including a thermometer, was heated in a bath of paraffin oil, which held an immersion heater connected to an adjustable potentiometer. The heating rate was 10 °C/min,

i.e. the same heating rate as in the DSC-experiments. The heating temperature for each starch-water mixture was chosen according to Figure 1. The gel was allowed to cool to room temperature (22°C), which took about 15 min. The gel was then removed from the test tube by the help of a spatula and placed on the plate in the stress relaxation instrument.

2.2.5 Stress Relaxation Measurements

The stress relaxation measurements were made with a cone-plate instrument, which is described in detail elsewhere [9]. The radius of the cone-plate system used was 1.23 cm and the cone-plate angle was 0.169 radians. The starch-gel was placed on the plate and the cone was lowered into the gel. Expelled materials were trimmed off with a knife. The sample at the edge of the cone-plate system was covered by silicone oil to prevent drying. The sample was then allowed to rest for 15 min. During this time the cone axis was free to rotate, so that some recovery motion might occur. The base plate was then rotated so that the lever on the cone axis came into contact with the pressure transducer. The time between the interruption of the heating of the gel and the application of the shear strain to the gel was the same for all gels (40 min), in order to reduce the effect of aging. All measurements were made at 22°C. The shear strains applied are given in Table 1. The output signal of the transducer was interfaced to a micro-computer (ABC 80 Luxor, Sweden), by an analog to digital converter. After completion of an experiment the relative stress $\tau(t)/\tau_0$ versus the natural logarithm at time was displayed on a digital plotter (Houston Instrument, Division of Bausch, Texas, USA). The stress relaxation was measured from 0.02 s to 400–1 000 s. The parameters calculated were the relaxation modulus (G) and the half relaxation time ($T_{1/2}$).

3 Results

3.1 Starch-Water Ratio and Temperature

In a previous study [2] the thermal transitions of wheat starch at different water contents were studied by DSC. These results were used to select the most interesting water contents of the gels for the stress-relaxation experiments in this investigation. The water contents of the samples were chosen to be 80, 60, 50 and 30% (w/w). The corresponding DSC-thermograms are given in Figure 1. The influence of the third thermal transition was not investigated since this transition is reversible [3] and thus impossible to study with the present method. At a water content of 80% (w/w) the gelatinization endotherm is thus the only endotherm observed on the DSC-thermogram in the investigated temperature interval. The temperatures to which the starch-water mixtures were heated prior to the stress-relaxation experiments are indicated by arrows on the DSC-thermogram in Figure 1. The temperatures were chosen so that gels with different degrees of gelatinization were obtained.

At intermediate water contents of 50 and 60% (w/w) the DSC-thermograms (Fig. 1) contain both the gelatinization endotherm and the second endotherm. At the water content of 30% (w/w) the amount of water is too low for gelatinization and the DSC-thermogram does not show any peak.

In Table 1 the starch-to-water ratios and temperatures investigated are listed. The shear strains used in the stress-relaxation experiments at different starch-to-water ratios are also included in this table. The same shear strains could not be used throughout all experiments since some gels were too stiff to give measurable results for the same shear strain as used for soft gels.

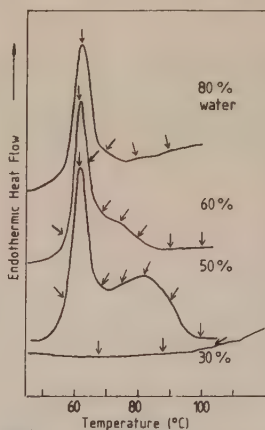


Figure 1. DSC-thermograms for wheat starch at different water contents. The arrows indicate the temperatures the gels were heated to prior to stress-relaxation measurements.

Table 1. Heating Temperatures and Shear Strains at Different Water Content.

Water-content (Weight-%)	Heating temperature (°C)	Shear strain
80	62.5, 70, 80 and 90	0.3
60	57, 61, 64, 68.5, 74, 80, 90 and 100	0.3
50	57, 61, 68.5, 75, 82, 90 and 100	0.1
30	22, 68, 88 and 104	0.1

The stress relaxation curves for starch-gels with 80% (w/w) water, heated to the temperatures given in Table 1, are shown in Figure 2. The reproducibility is indicated by solid vertical bars on the curve for the gel heated to 80°C. The bars represent the scatter between 3 individual experiments. The reproducibility was the same for all starch-water ratios and temperatures tested. The half relaxation time and the relaxation modulus for each temperature are given in Table 2. Both $T_{1/2}$ and G are higher for the gel heated to 70°C compared to the gel heated to 62.5°C. At 70°C gelatinization is almost complete (Fig. 1), and the values for $T_{1/2}$ and G are almost the same for the gels heated to 80°C and 90°C. The residual stress at long times seems to be highest for the gel heated to the lowest temperature, i.e. 62.5°C.

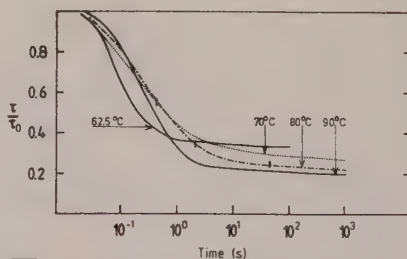


Figure 2. Stress-relaxation curves for wheat starch gels with 80% (w/w) water. The temperature to which each gel has been heated is given in the figure. The shear strain was 0.3.

Table 2.
Half Relaxation time ($T_{1/2}$) and Relaxation Modulus (G) for Starch-Water Mixtures of Different Water Content Heated to Different Temperatures.

Water content (Weight-%)	Heating temperature (°C)	$T_{1/2}$ (s)	G (Pa)
80	62.5	0.2	3.2×10^3
	70	1.1	1.3×10^4
	80	0.7	1.7×10^4
	90	1.2	1.7×10^4
	90	0.1	1.0×10^4
60	57	0.1	1.0×10^4
	61	0.3	4.6×10^4
	64	0.3	5.7×10^4
	68.5	0.7	5.7×10^4
	74	0.6	6.2×10^4
	80	7.4	4.7×10^4
	90	8.7	3.0×10^4
	100	10.5	2.8×10^4
50	57	0.6	1.2×10^5
	61	0.8	1.9×10^5
	68.5	1.7	2.2×10^5
	75	61	1.3×10^5
	82	106	1.1×10^5
	90	735	0.9×10^5
	100	786	0.5×10^5
	100	786	0.5×10^5
30	22	0.5	5.0×10^4
	68	6.5	4.5×10^4
	88	1.8	3.2×10^4
	104	2.3	2.6×10^4

At intermediate water contents, for example at 50% (w/w), the influence of the heating temperature on the relaxation properties of the gel is different. The stress relaxation curves are given in Figure 3, and the calculated parameters are given in Table 2. At 50% (w/w) water there is a similar increase in $T_{1/2}$ and G during the gelatinization as at 80% (w/w) water. However, the G -values for the gels heated to higher temperatures are lower. The maximum relaxation modulus is obtained at 68.5°C ($G = 2.2 \times 10^5$ Pa). The higher the heating temperature of the gel, the greater is the $T_{1/2}$ -value. The increase in $T_{1/2}$ is not linear, however. The greatest change is found when the gel heated to 90°C is compared to the gel heated to 82°C. The G - and $T_{1/2}$ -values for the gel heated to 100°C are almost the same as for the gel heated to 90°C. The higher temperature the gels with 50% (w/w) water are heated to, the higher is the value of the residual stress at long times. The principal appearance of the stress relaxation curves for gels with 60% (w/w) water is the same as for the gels with 50% (w/w) water. Thus only the calculated values of $T_{1/2}$ and G are given (Table 2).

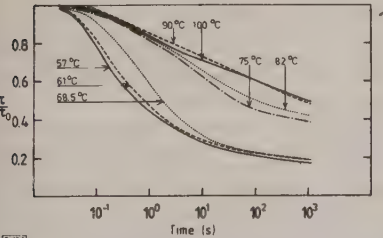


Figure 3. Stress relaxation curves for wheat starch gels with 50% (w/w) water. The temperature to which each gel has been heated is given in the figure. The shear strain was 0.1.

The relaxation curves for starch-water mixtures with only 30% (w/w) water are shown in Figure 4, and the values of $T_{1/2}$ and G are given in Table 2. At this high starch content, it was also possible to measure the stress relaxation on the starch-water mixture without heating. $T_{1/2}$ is longer and G is smaller for the heated starch-water mixtures. If the stress-relaxation curves are extrapolated to longer times, the stress extrapolates to zero.

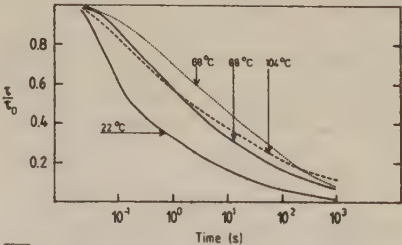


Figure 4. Stress relaxation curves for wheat starch gels with 30% (w/w) water. The temperature to which each gel has been heated is given in the figure. The shear strain was 0.1.

3.2 Particle Size Effect

The particle size distributions of the two starch preparations were measured on a Coulter counter and the results are given in Figure 5. More of the small granules were extracted with the prolonged washing procedure (starch preparation II in Fig. 5). The protein content and the fraction of damaged granules, however, are the same for the two preparations (Table 3).

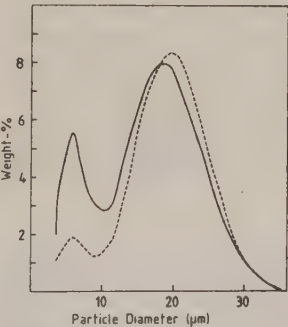


Figure 5. Particle size distributions for the two starch preparations. --- Starch preparation I, — Starch preparation II.

Table 3.
Properties of the Two Starch Preparations Obtained (see also Fig. 5).

	Starch preparation I	Starch preparation II
Protein, d. b. (%) ¹⁾	0.2	0.2
Damage (%) ²⁾	2	3
Gelatinization enthalpy (cal/g) ³⁾	2.5 ± 0.1	2.3 ± 0.1
Gelatinization temperature (°C) ³⁾	60.4 ± 0.5	60.5 ± 0.5

¹⁾ Kjeldahl-N $\times 5.7$.
²⁾ Granules coloured by Congo-red were counted in microscope.
³⁾ Determined by DCS for a starch-water mixture with 60% (w/w) water.

The influence of particle size on the relaxation properties of a starch gel was investigated for gels with 60% (w/w) water. In these experiments the two starch preparations were compared, in all other experiments preparation II (Fig. 5) was used. G and $T_{1/2}$ for the two types of starch-gels heated to different temperatures are given in Figure 6. The G -values for the gels with a greater fraction of large granules are lower at all temperatures except at 90°C. At this temperature the G -values are almost the same (about 2.8×10^4 Pa) for both starch preparations. For the starch with proportionally more large granules this value is obtained already at 80°C and for gels heated to higher temperatures this value does not change. The half relaxation times are almost the same for the two starches, when the gels are heated to 74°C or below. For gels heated to temperatures higher than 74°C, $T_{1/2}$ is longer for the gels with a greater fraction of small granules.

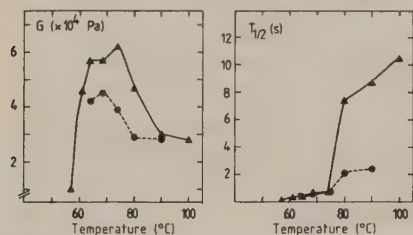


Figure 6. Relaxation modulus (G) and half relaxation time ($T_{1/2}$) for wheat starch gels with 60% (w/w) water after heating to different temperatures. --- Starch preparation I (see Fig. 5), — Starch preparation II (see Fig. 5).

According to DSC-measurements small wheat starch granules have higher gelatinization temperature than large granules, whereas the gelatinization enthalpy is the same for both types of granules [10]. Hence, DSC-measurements were used to check if the difference in particle size distribution caused changes in these parameters. From the results given in Table 3 it is evident that the gelatinization temperature and gelatinization enthalpy are the same for the two starch preparations. Thus, it seems possible to assign the differences in relaxation modulus and half relaxation time to differences in the particle size alone.

4 Discussion

During the gelatinization of starch the stress relaxation modulus and the half relaxation time increase (Table 2). When the starch is heated in excess of water, $T_{1/2}$ and G do not change for gels heated to temperatures above complete gelatinization. When the starch is heated in the presence of limited amounts of water, G is lower and $T_{1/2}$ is longer for gels heated to temperatures above the temperature interval for gelatinization. A maximum G -value is thus obtained when this temperature interval is passed, whereas a maximum $T_{1/2}$ -value is not obtained until also the second thermal transition, according to the DSC-thermogram, is complete.

In order to understand the present results for the stress relaxation of concentrated starch gels we shall discuss a model composite system of a viscoelastic continuous phase and a disperse phase of particles. We shall consider how the viscoelastic properties of the composite system may depend on

- the rigidity of the dispersed particles,
- the viscoelasticity of the continuous phase,
- the adhesion between the dispersed and continuous phases.

The stress relaxation modulus is increased by the addition of a filler, which is rigid compared to the continuous phase. The shear modulus of e.g. a polyurethane rubber filled with sodium chloride increases by an order of magnitude when the filler concentration is increased from 0 to 59.8 vol.-% [11]. Similarly the shear relaxation modulus of wheat flour dough is an order of magnitude larger than the relaxation modulus of the gluten of the same flour [12]. The addition of a rigid filler is also normally accompanied by an increase in the relaxation rate. This is attributed to the appearance of new mechanisms for viscous flow, such as particle-polymer friction and also excess damping in the polymer near the interface [13]. The addition of soft particles may, on the other hand, decrease the relaxation modulus and decrease the relaxation rate, i.e. increase the half relaxation time.

Changes in viscoelastic properties of the continuous phase are normally directly reflected in the properties of the composite system, e.g. an increase in elasticity of the continuous phase gives a corresponding increase in the elasticity of the composite.

In a composite with no adhesion between filler and continuous phase, mainly a viscous contribution is obtained by adding the filler, i.e. the addition of filler particles increases the relaxation rate. When the adhesion between filler and continuous phase is good, the relaxation time may be the same for the composite material as for the pure continuous phase, whereas the relaxation modulus increases.

A starch-gel may be regarded as a composite material, where the starch granules act as filler in a continuous phase consisting of a solution or gel of polysaccharides in water. It is necessary, however, to take into consideration that when a starch-water mixture is heated both the rigidity of the filler, the viscoelasticity of the continuous phase, and the adhesion between the dispersed and continuous phases may change all at the same time. These changes may affect the rheological properties of the starch gel in different directions.

If the starch granules are regarded as rigid fillers compared to the continuous phase, an increase in relaxation modulus would be expected when the amount of filler is increased. This is also the case if the gel with 80% (w/w) water heated to 62.5°C ($G = 3.2 \times 10^3$ Pa) is compared to the gel with 60% (w/w) water heated to 61°C ($G = 4.6 \times 10^4$ Pa). However, in this case the increase in G -value is not accompanied by an increase in the relaxation rate, $T_{1/2}$ is almost the same for both gels.

For all gels with water enough for gelatinization to occur, G increases during the gelatinization. There is also a small increase in $T_{1/2}$. It seems unlikely that the starch granules would become more rigid during the gelatinization and thus explain the increase in G . A necessary condition for the formation of a gel is the leaking of amylose molecules out from the starch granules [14]. The increase in G and $T_{1/2}$ during the gelatinization could thus be explained by an increased elasticity of the continuous phase. During the leaking process it is also possible that the adhesion between the dispersed and continuous phases will change. The adhesion will probably increase in strength, since a lesser adhesion would decrease $T_{1/2}$, and such an effect is not observed.

Gels with 60% and 50% (w/w) water, obtained by heating to temperatures high enough for the second thermal process to occur, have lower G -values and higher $T_{1/2}$ -values. As discussed above, in composite materials this change in rheological properties is obtained when a soft filler is added to the continuous phase. The second thermal transition could thus be interpreted as a process which makes the starch granules softer.

For gels with 30% (w/w) water, no endotherms are observed on the DSC-thermogram. The changes in the values of G for the gels heated to different temperatures do not follow the pattern for the gels where gelatinization occurs. Instead the maximum value of G is obtained at room temperature. The lack of residual stress after long relaxation time indicates that no true gel is formed.

The size of the starch granules seems to affect the relaxation properties markedly. A larger fraction of small granules increases both the relaxation modulus and the half relaxation time. This is generally observed for composite materials [15] and might be due to a better packing of the starch granules. Different water binding capacities of small and large granules could also contribute to this effect.

Acknowledgement

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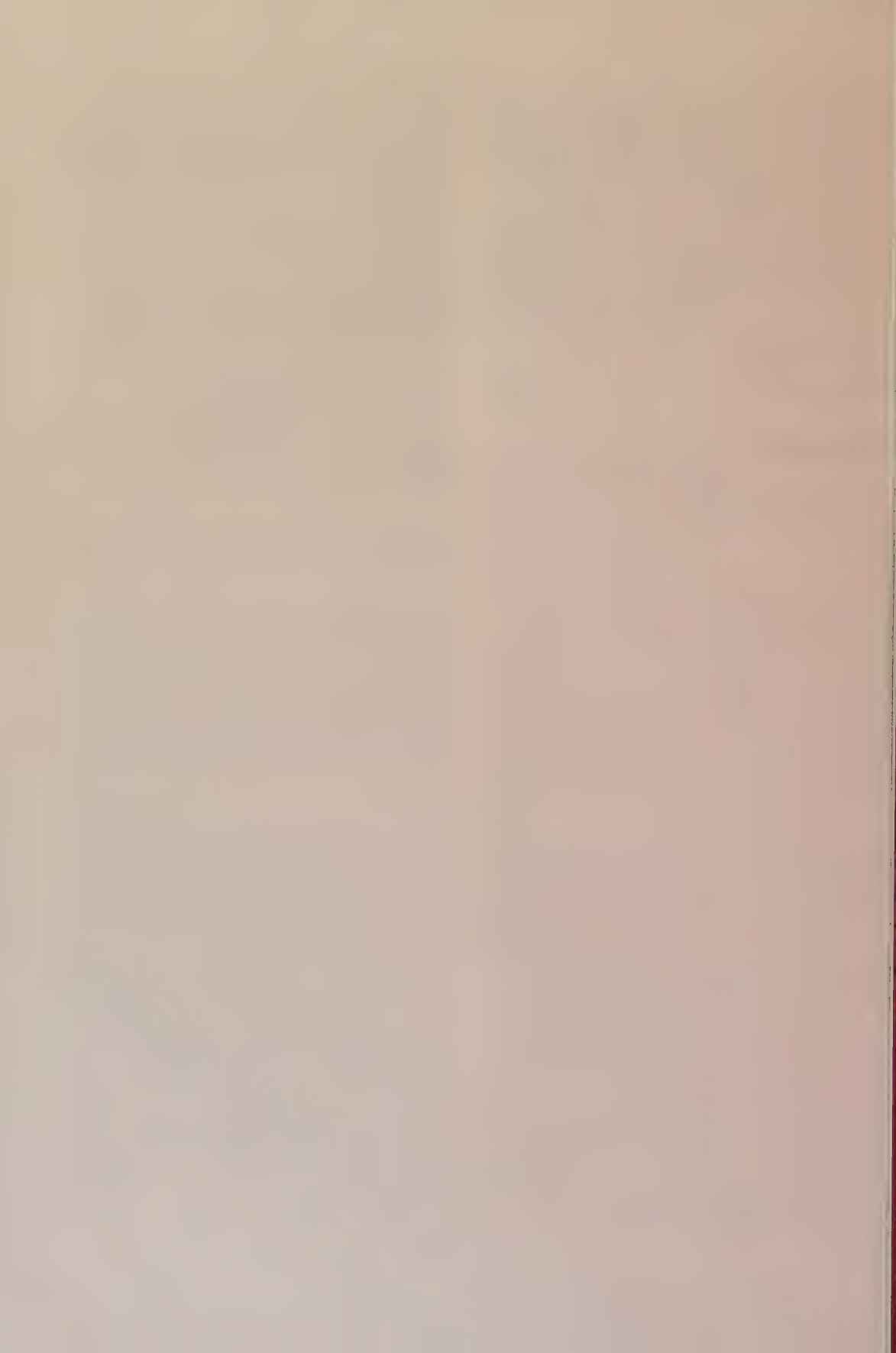
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VI

DIFFERENTIAL SCANNING CALORIMETRY STUDIES ON

THE SYSTEM GLUTEN-WHEAT STARCH

I. EFFECT OF GLUTEN ON THE GELATINIZATION OF WHEAT STARCH

by

A.-C. Eliasson

DIFFERENTIAL SCANNING CALORIMETRY STUDIES ON THE SYSTEM
GLUTEN-WHEAT STARCH

I. Effect of Gluten on the Gelatinization of Wheat-Starch

by

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SUMMARY

The influence of gluten on wheat starch gelatinization was studied by differential scanning calorimetry (DSC). The presence of gluten decreased the starch gelatinization enthalpy and increased the gelatinization temperature. The values obtained in presence of gluten were compared with the pure starch-water system, and the amount of water associated with gluten during the heating was calculated. It was found that water was transported from gluten to starch during the whole heating period.

INTRODUCTION

The gelatinization behaviour of starch is known to depend on the water content¹⁻³. Food ingredients which influence the water activity, e.g. sugar and salt, thus affect the gelatinization of starch⁴. Also proteins are known to influence the starch gelatinization⁵, but this can be caused in a different way, such as separation of water into two phases - a protein aggregate phase and a starch phase. In a complex food system with competition between the components for the available water, e.g. in a dough, the degree of starch gelatinization will thus depend on the distribution of water between the colloidal components in the system, and on the water activity in the corresponding aggregates.

In this investigation the effect of gluten on wheat starch gelatinization was studied by differential scanning calorimetry (DSC). In a previous study⁶, it was found that the enthalpies of transitions occurring in gluten during heating were negligible compared to the transitions in starch, and the peaks observed on the DSC-thermograms could thus be assigned to transitions occurring in the starch only. It should therefore be possible to compare the gelatinization of starch in the gluten-starch-water system with the pure starch-water system, and calculate the amount of water available for the starch when gluten is present.

EXPERIMENTAL

Materials

Starch was isolated from whole wheat grains according to the steeping procedure described by Meredith et al.⁷. The primary fraction was used after drying in the air at room temperature. Starch from the spring wheat Amy was used in all experiments.

Gluten was isolated from flour by washing dough with water in an automatic gluten washer (Falling Number, Stockholm, Sweden) until no more starch could be released from the gluten. The gluten was dried at 40°C in a vacuum oven, and milled to a fine powder. Gluten from Amy was used in all experiments except in one, where gluten from Maris Huntsman was used.

The water contents of starch and gluten were measured according to the AACC-method 44-15⁸.

Distilled - deionized water was used throughout all experiments.

Preparation of samples for DSC

200 mg starch and an amount of gluten to give a ratio of gluten (d.m.) to starch (d.m.) of 0.10, 0.20 or 0.40 were mixed dry in a test tube with a spatula. Water was added with a Hamilton syringe to give a water-to-starch ratio of 0.90. The amount of water in the starch and gluten samples was included in the total amount of water. The samples were thoroughly mixed with the spatula and about 8 to 12 mg of the "dough" were weighed into DSC sample pans. The time between the mixing of the "dough" and the heating in the DSC was about 3 hours.

DSC

The DSC used was a Perkin-Elmer DSC-2C. The scanning rate was $10^{\circ}\text{C}/\text{min}$, and the scan was started at 27°C and ended at 100°C . The sample pan was then immediately cooled to 27°C at a cooling rate of $320^{\circ}\text{C}/\text{min}$, and removed from the DSC. An empty sample pan was used as reference. The results given are the mean and standard deviation of 9 scans.

RESULTS AND DISCUSSION

The DSC-thermograms for doughs consisting of starch, water (0.90 g water per g starch) and varying amounts of gluten are given in Fig. 1.

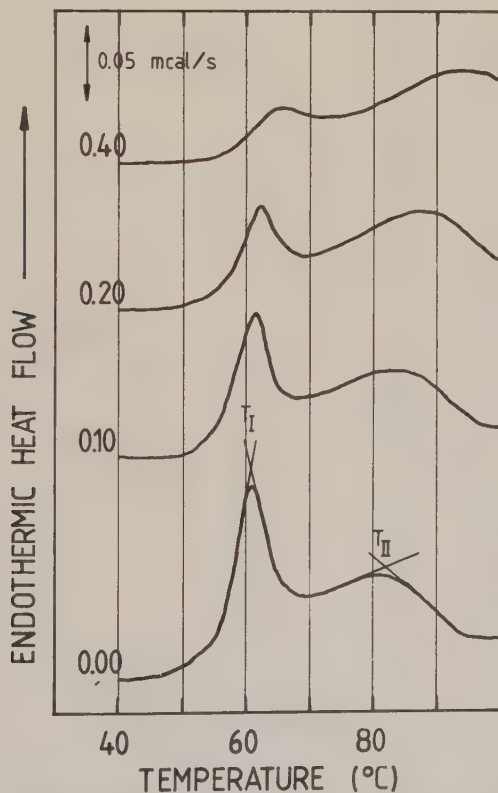


Fig. 1. The DSC-thermograms for heating a mixture composed of 0.90 g water per gram starch and the amount of gluten per gram starch indicated at each thermogram. The total amount of sample is, from top to below: 7.85, 8.27, 8.35 and 8.40 mg.

As was discussed above, the DSC-thermograms could be interpreted as showing the thermal behaviour of starch only, since DSC-thermograms of gluten have shown that enthalpies of the transitions which could be assigned to the gluten proteins were less than 0.1 cal/g gluten⁶. The contribution to the enthalpy from starch remaining in gluten was not corrected for since it was less than the experimental error in the enthalpy determinations.

Enthalpies

The enthalpies of the two transitions occurring in the gluten-starch-water system were calculated separately, and the results are given in Fig. 2.

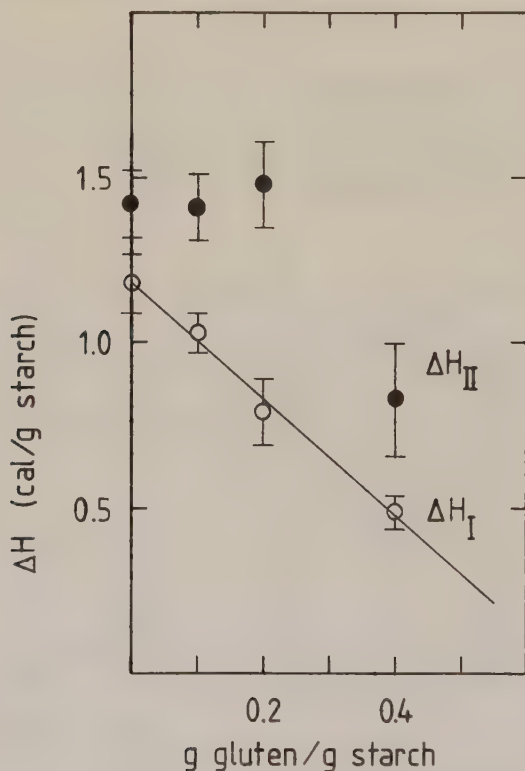


Fig. 2. ΔH_I and ΔH_{II} as a function of the gluten-to-starch ratio at a water content of 0.90 g water/g starch.

ΔH_I decreased with increasing gluten-to-starch ratio, whereas ΔH_{II} was almost independent of the gluten-to-starch ratio. The low value for the sample with 0.40 g gluten per g starch was, at least in part, due to the fact that heating was interrupted at 100°C, and in this sample the whole transition did not occur below 100°C (see Fig. 1).

Regression analysis of the results gave the following relation between ΔH_I and gluten-to-starch ratio.

$$\Delta H_I \text{ (cal/g starch)} = - 1.76 \cdot \frac{\text{g gluten}}{\text{g starch}} + 1.18$$

The correlation coefficient was -0.9957. If this relationship is extrapolated to higher gluten-to-starch ratios, 0.67 g gluten per g starch at this water content totally should inhibit the starch gelatinization. In a starch-water system ΔH_I decreases as the amount of water decreases (cf. ref. 1-3). The decrease of ΔH_I in the gluten-starch-water dough, could thus be interpreted as a decreasing amount of water available for the starch. The relation between ΔH_I and g water/g starch in the pure starch-water system was used to calculate the amount of water per gram starch in the dough, and the results are given in Table I.

TABLE I

The amount of water available for the starch during the heating of a dough consisting of 0.90 g water/g starch and varying amounts of gluten

$\frac{\text{g gluten}}{\text{g starch}}$	$\frac{\text{g water}}{\text{g starch}}$	
	Transition I	Transition II
0.00	0.91	0.93
0.10	0.83	0.88
0.20	0.76	0.83
0.40	0.62	0.74

From these results the water associated with gluten during the starch gelatinization was calculated to 0.71 g water/g gluten.

In the pure starch-water system, ΔH_{II} increases when the water content decreases (cf. ref. 1-3). As the ΔH_{II} -values in the gluten-starch-water system seemed to be independent of the gluten-to-starch ratio (see Fig. 2), it might be concluded that the amount of water available for the starch during the last part of the heating, was the same at all gluten-to-starch ratios. However, the changes in T_{II} , which will be discussed below, did not support

such an interpretation. A difficulty in using ΔH_{II} to calculate the amount of water available for the starch, is that ΔH_{II} as a function of water content has a maximum, i.e. two different water-to-starch ratios may result in the same value of ΔH_{II} .

Temperatures

Both T_I and T_{II} depended on the gluten-to-starch ratio as illustrated in Fig. 3.

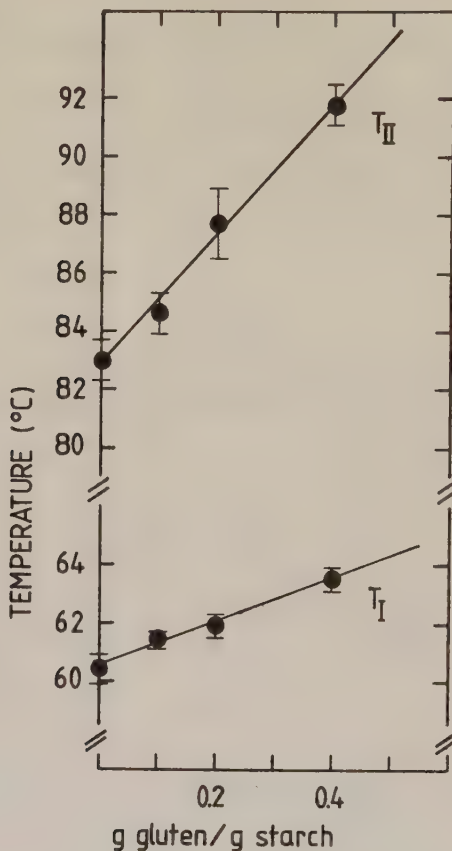


Fig. 3. T_I and T_{II} as a function of the gluten-to-starch ratio at a water content of 0.90 g water/g starch.

Regression analysis of the results gave the following relations between transition temperatures and the gluten-to-starch ratio:

$$T_I (^{\circ}\text{C}) = 60.48 + 7.54 \times \frac{\text{g gluten}}{\text{g starch}}$$

and

$$T_{II} (^{\circ}\text{C}) = 82.82 + 22.60 \times \frac{\text{g gluten}}{\text{g starch}}$$

The correlation coefficients were 0.9958 and 0.9956, respectively.

In the pure starch-water system, T_I is independent of the water-to-starch ratio (cf. ref. 1-3). The increase in T_I may be due to a delay in the diffusion of water into the starch granules because of the presence of gluten on the surface of the starch granules. A higher starch gelatinization temperature in wheat flour compared to the isolated starch has been observed by others⁹.

The increase in T_{II} with increasing gluten-to-starch ratio might be due to a decreasing amount of water available for the starch, since in the pure starch-water system T_{II} increases with decreasing water-to-starch ratios. When the T_{II} -values obtained in the gluten-starch-water system were compared to the pure starch-water system, the amount of water per g starch given in Table I was obtained. The calculated value of the amount of water per gram starch when no gluten was added was somewhat higher than the expected value. The difference is caused by the uncertainty in the calibration curves (the standard deviation in ΔH_I was less than 0.05 cal/g and in T_{II} about 1 $^{\circ}\text{C}$), and might be regarded as a measure of the accuracy in the calculations of the amount of water available to the starch. From the results in Table I the water associated with gluten during the second transition in the starch was calculated to be 0.48 g water/g gluten.

The distribution of water between starch and gluten during the heating may thus change as described in Table II. The results show that about 11% of the water still was associated with the gluten when the heating was interrupted. The transport of water from gluten to starch is considered to continue also during the storage of bread¹⁰, and thus play a significant role in the staling.

The results given above demonstrate how the gelatinization of starch, and thus the properties of the whole starch-containing system, can be altered by the presence of components which compete with the starch for the available water. The doughs discussed so far were reconstituted from starch and gluten from wheat variety Amy. An experiment was carried out to investigate

TABLE II

Distribution of water between starch and gluten during the heating of a dough consisting of 0.20 g gluten/g starch and 0.90 g water/g starch

	% water associated with	
	starch	gluten
During transition I	84	16
During transition II	89	11

whether the distribution of water between starch and gluten could be affected by quality differences between varieties. The results are given in Table III, and these few results do not suggest that the thermal behaviour of the dough varies due to the wheat variety, as far as gluten is concerned. Also the starch variety could be expected to affect the water distribution between starch and gluten. However, this influence is not possible to study by a simple exchange of the starch, since the relation between ΔH_I and the water content, which is used for the calculation of the water distribution, is known to depend on the wheat starch variety¹¹. As a continuation of this work the influence of the pentosans on the gelatinization properties of starch will be studied.

TABLE III

Influence of gluten variety on the starch gelatinization in a dough consisting of 0.20 g gluten/g starch and 0.90 g water/g starch

Starch	Gluten	ΔH_I	ΔH_{II}	T_I	T_{II}
		(cal/g starch)			(°C)
Amy	Amy	0.79 ± 0.10	1.48 ± 0.13	61.0 ± 0.4	87.7 ± 1.2
Amy	Maris Huntsman	0.82 ± 0.06	1.46 ± 0.09	61.3 ± 0.3	87.5 ± 0.6

Acknowledgements

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VII

DIFFERENTIAL SCANNING CALORIMETRY STUDIES ON THE SYSTEM GLUTEN-WHEAT STARCH

II. EFFECT OF GLUTEN AND SURFACTANTS ON THE CRYSTALLIZATION OF WHEAT STARCH DURING AGEING OF THE STARCH GEL

by

A.-C. Eliasson

DIFFERENTIAL SCANNING CALORIMETRY STUDIES ON THE SYSTEM
GLUTEN-WHEAT STARCH

II. Effect of Gluten and Surfactants on the Crystallization
of Wheat Starch during Ageing of the Starch Gel

by

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SUMMARY

The crystallization of wheat starch in the presence of gluten and surfactant during storage was measured by differential scanning calorimetry (DSC). It was found that the crystallization of starch during ageing is possible to decrease by the addition of gluten. However, this addition may also give the opposite effect, since the addition of gluten may decrease the amount of water available to the starch, and thus increase the starch crystallization. Furthermore, it was found that the Avrami-equation could not be used to analyse the result. The Avrami-exponent was less than unity, and the findings in this investigation thus indicate that the Avrami-model may be too simple to describe the starch crystallization.

The gelatinization enthalpy was decreased, and the gelatinization temperature was increased by the addition of a surfactant. However, both complexing of amylose and retardation of gelatinization occurred at higher amounts of surfactant than was necessary to delay the starch crystallization.

INTRODUCTION

The crystallization of starch is one important factor in the staling of bread¹⁻⁵. This crystallization process has been followed with differential thermal analysis⁶ (DTA) and differential scanning calorimetry^{7,8} (DSC). When

an aged starch gel, or the crumb from staled bread, is reheated, an endothermic transition at about 50-70°C occurs, which is associated with the amylopectin fraction of the starch⁷.

The effects of proteins^{9,10}, pentosans^{11,12} and surfactants¹³ on the staling of pure wheat starch gels and of bread have been studied by measuring crumb firmness. The direct effects of such components on the starch crystallization have, however, been neglected, apart from a recent report concerning the effect of pentosans on the starch crystallization⁸.

As a continuation of a previous work on the effect of gluten on the gelatinization of wheat starch¹⁴, the effect of gluten on the ageing of starch gels is reported here. Surfactants were added to the gluten-starch-water system, and the influence of the surfactant on starch gelatinization and crystallization was also studied.

EXPERIMENTAL

The starch and gluten used, and the preparation procedures have been described elsewhere¹⁴. The surfactant used was sodiumstearoyl lactylate (SSL) (Artodan SP50 from A/S Grindstedvaerket, Denmark). SSL was mixed with water (1:10) and allowed to swell during one day to form a dispersion of the gel-state. Before the addition to the gluten-starch mixture, the SSL-gel was diluted with water to give the proper concentration of both SSL and water in the final sample. The preparation of samples and the DSC-measurements have been described earlier¹⁴.

The same samples as were used to study the effect of gluten on the starch gelatinization¹⁴ were also used to study the starch crystallization during storage. When the sample pans had been heated to 100°C, they were rapidly cooled to 27°C and removed from the DSC. The sample pans were stored at 21°C during 1, 3 and 7 days, and then reheated in the DSC at a heating rate of 10°C/min. A few samples were stored for longer times (18 and 43 days). The results given are the mean and standard deviation of three measurements.

RESULTS AND DISCUSSION

Starch

In an initial series of experiments the ageing of starch-water gels was studied. ΔH_c (the enthalpy of melting crystallized starch) as a function of

storage time is shown in Fig. 1 for starch-water gels with 0.90 g water/g starch. The transition peak temperature (T_g) decreased during the first seven days of storage (from 60.6°C to 58.1°C) and increased thereafter (62.8°C at 43 days of storage).

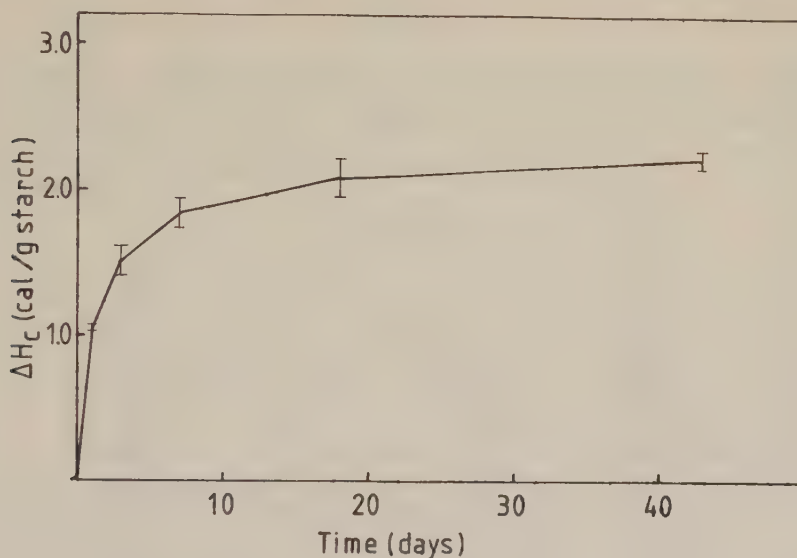


Fig. 1. The increase in ΔH_c with time for wheat starch gels containing 0.90 g water/g starch.

To interpret the results from measurements of staling and starch crystallization the Avrami-model has been used¹⁻¹². The fraction of uncrystallized material (θ) is related to time (t):

$$\theta = \exp (-k t^n)$$

where k is a growth parameter, and n is an integer varying from 1 to 4 that is characteristic of the mode of nucleation. Measurements of crumb firmness⁹⁻¹² and DTA-measurements⁶ have shown the Avrami exponent (n) to be 1, and based on this, it has been suggested that the mechanism of starch crystallization is instantaneous nucleation followed by rod-like growth of crystals. However, in a DSC-study⁸, the Avrami-exponent was found to be less than unity (0.4 - 0.8), and in a recent study¹⁵, where dynamic rheological properties of stored starch gels were measured, n was found to be 2.

The results obtained in this investigation for the starch-gels containing 0.90 g water/g starch were analysed according to the Avrami-model. The ΔH_c

at 43 days (2.22 cal/g starch) was taken as the limiting value. The analysis gave a value of 0.5 for the Avrami-exponent, and 1.54 days for $1/k$. This result is in good agreement with the DSC-study discussed above⁸, and thus suggests that the Avrami-model may be too simple to accurately describe the starch crystallization.

The effect of the water content on the crystallization of starch was studied for starch-gels stored three days, and the result is given in Fig. 2.

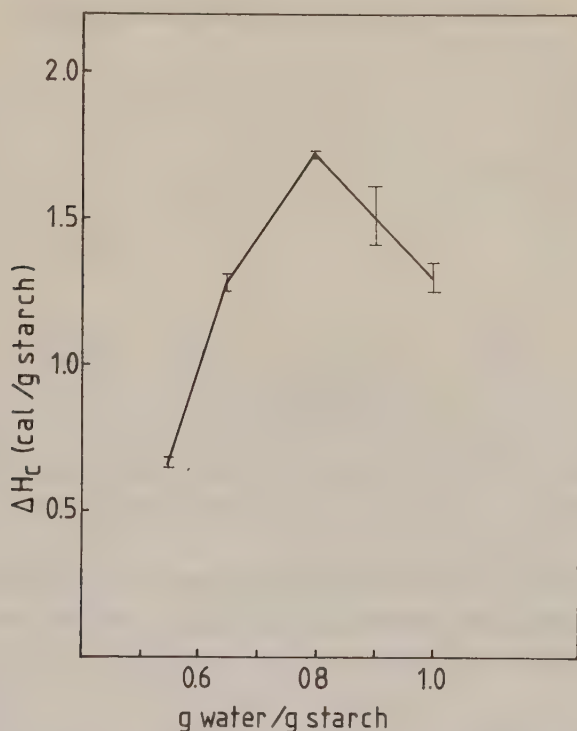


Fig. 2. The dependence of ΔH_c on the water content for wheat starch gels stored three days.

This result is in good agreement with what has been reported earlier⁸. The dependence of ΔH_c on the water content thus means that the water available for the starch will influence the crystallization. Depending on the initial amount of water available, a substance which increases the amount of water available for the starch could either increase or decrease ΔH_c .

Gluten-Starch

When gluten was mixed into the wheat starch gel at the water content of 0.90 g water/g starch, ΔH_c changed as shown in Fig. 3. The addition of gluten thus decreased the starch crystallization except for the gel with 0.20 g gluten/g starch stored one day. T_c increased with increasing amount of gluten. For the gels stored one day, T_c was 60.6°C without added gluten, and 62.1°C with 0.40 g gluten/g starch. After seven days the difference had increased, and T_c for gels without gluten was 58.1°C, and 61.6°C for gels with 0.40 g gluten/g starch.

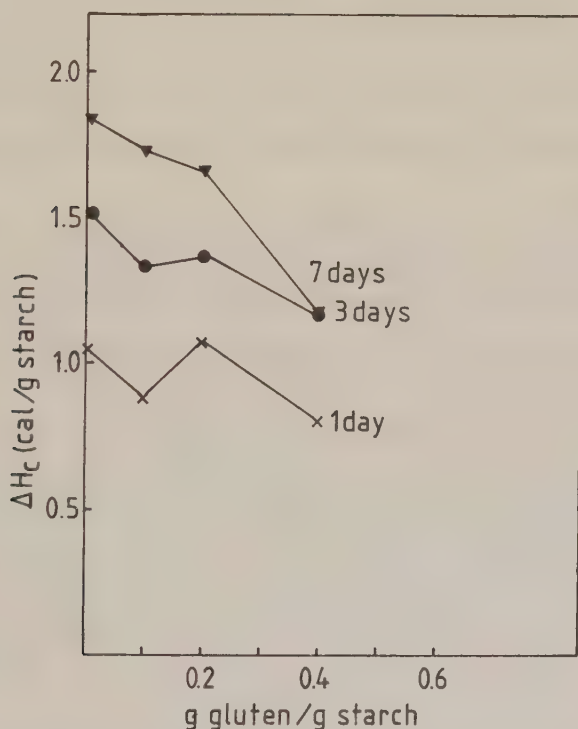


Fig. 3. The influence of gluten on the starch crystallization. The water content is 0.90 g water/g starch.

A decreasing effect of protein on the staling of bread and starch gels has been reported in crumb firmness studies^{9,10}.

In the previous work¹⁴, the effect of gluten on the starch gelatinization was interpreted as an effect due to a smaller amount of water available for the starch in the presence of gluten. This might also be one explanation of the effect of gluten on the starch crystallization, i.e. the amount of water

available to the starch is changed by the gluten and ΔH_C will increase or decrease according to Fig. 2. The amount of water available to the starch at the end of the heating at the three gluten levels was calculated to be 0.88, 0.83 and 0.74 g water/g starch¹⁴, respectively. According to Fig. 2 an increase in ΔH_C would thus be expected. Such an increase was not observed, except for the gel containing 0.20 g gluten/g starch stored one day. The influence of gluten on the amount of water available for the starch does not account for the effect of gluten on the starch crystallization. Another effect of the gluten may be that the presence of a gluten film on the starch granules affect the amylose/amylopectin ratio in the granules, which could influence the crystallization process.

The results in Fig. 3 were analysed according to the Avrami-model. For gels containing gluten, the Avrami-exponent was also found to be less than unity.

The influence of the wheat variety on the starch crystallization was also studied. In the experiments discussed so far, gluten and starch from the wheat variety Amy were used. Experiments were carried out where the Amy gluten was replaced by gluten from the wheat variety Maris Huntsman, and the Amy starch was replaced by starch from Maris Huntsman. The results are given in Table I. The gluten quality seemed not to influence the starch crystallization. This has also been reported for crumb firmness measurements⁹. The starch, on the other hand, had a great influence on the crystallization process. ΔH_C for the gels containing starch from Maris Huntsman was lower at all storage times. T_C was influenced by the kind of starch (T_C was lower for gels containing the starch from Maris Huntsman), but not by the gluten.

TABLE I

The influence of gluten and starch variety on the starch crystallization. 0.20 g gluten/g starch and 0.90 g water/g starch

Gluten	Starch	ΔH_C (cal/g starch)		
		1 day	3 days	7 days
Amy	Amy	1.07 $\pm$ 0.05	1.36 $\pm$ 0.10	1.66 $\pm$ 0.05
Maris Huntsman	Amy	1.02 $\pm$ 0.05	1.42 $\pm$ 0.04	1.58 $\pm$ 0.03
Amy	Maris Huntsman	0.69 $\pm$ 0.08	1.29 $\pm$ 0.08	1.48 $\pm$ 0.04

Gluten-Starch-Surfactant

The surfactant used in this investigation was sodium-stearoyl lactylate (SSL), which is known to have a good anti-staling effect¹⁵. SSL was added to a gluten-starch mixture with 0.20 g gluten/g starch, and the water content was 0.90 g water/g starch. The effect of SSL was studied at two levels, 0.006 g SSL/g starch and 0.023 g SSL/g starch.

The influence of SSL on the gelatinization of starch is shown in Table II. The presence of SSL decreased ΔH_I and ΔH_{II} , and increased T_I . However, at the amount of SSL corresponding to practical use in bread (0.006 g SSL/g starch), the effect on ΔH_I and T_I was small. The effect of SSL on the crystallization of starch is shown in Fig. 4. Although the high amount of SSL influenced the gelatinization of the starch, it did not decrease ΔH_C more than the low amount of SSL did. T_C was higher for gels containing SSL.

TABLE II

Influence of SSL on the gelatinization of wheat starch.
0.20 g gluten/g starch and 0.90 g water/g starch.

$\frac{\text{g SSL}}{\text{g starch}}$	ΔH_I (cal/g starch)	ΔH_{II}	T_I (°C)	T_{II}
0.000 ^a	0.79 ± 0.10	1.48 ± 0.13	61.9 ± 0.4	87.7 ± 1.2
0.006	0.76 ± 0.05	1.19 ± 0.10	62.1 ± 0.1	86.2 ± 0.6
0.023	0.63 ± 0.06	1.11 ± 0.05	63.2 ± 0.3	88.1 ± 0.7

^aFrom ref. 14.

The gels stored for seven days were reheated to temperatures high enough to observe the transition due to the amylose-lipid complex^{16,17}. The enthalpy of this transition was proportional to the amount of SSL added, and increased from 0.58 cal/g starch without SSL to 1.10 cal/g starch at the highest amount of SSL added. It could thus be concluded that the added SSL complexed with the amylose. However, although apparently more complexes were formed at the higher amount of SSL added, this did not influence the starch crystallization during the storage of the gels. The mechanism behind the anti-staling effect of SSL is therefore not only amylose complexing. Work in progress¹⁸ indicates that the complex forms a barrier, which may influence the water

distribution between the continuous gluten phase and the dispersed starch granules.

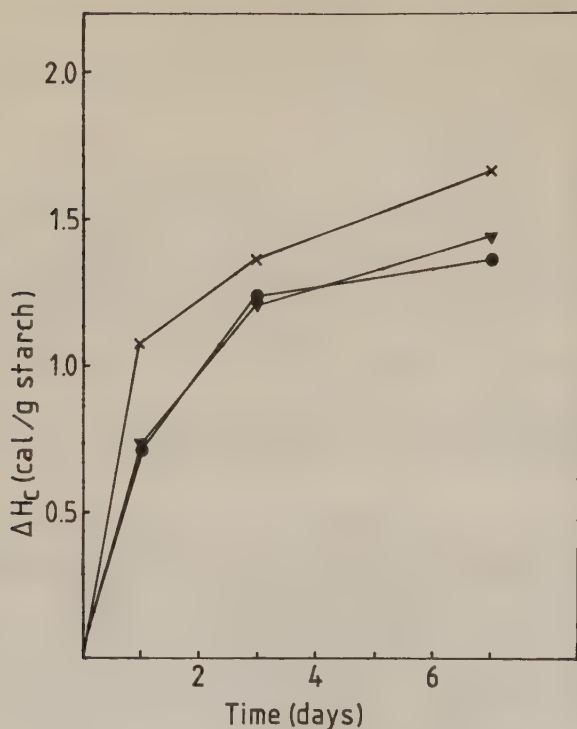


Fig. 4. The increase in ΔH_c with time for wheat starch gels with and without sodium stearoyl-lactylate (SSL). The starch gels consist of 0.90 g water/g starch, 0.20 g gluten/g starch and $\times$ — $\times$ no SSL, $\bullet$ — $\bullet$ 0.006 g SSL/g starch, $\blacktriangledown$ — $\blacktriangledown$ 0.023 g SSL/g starch.

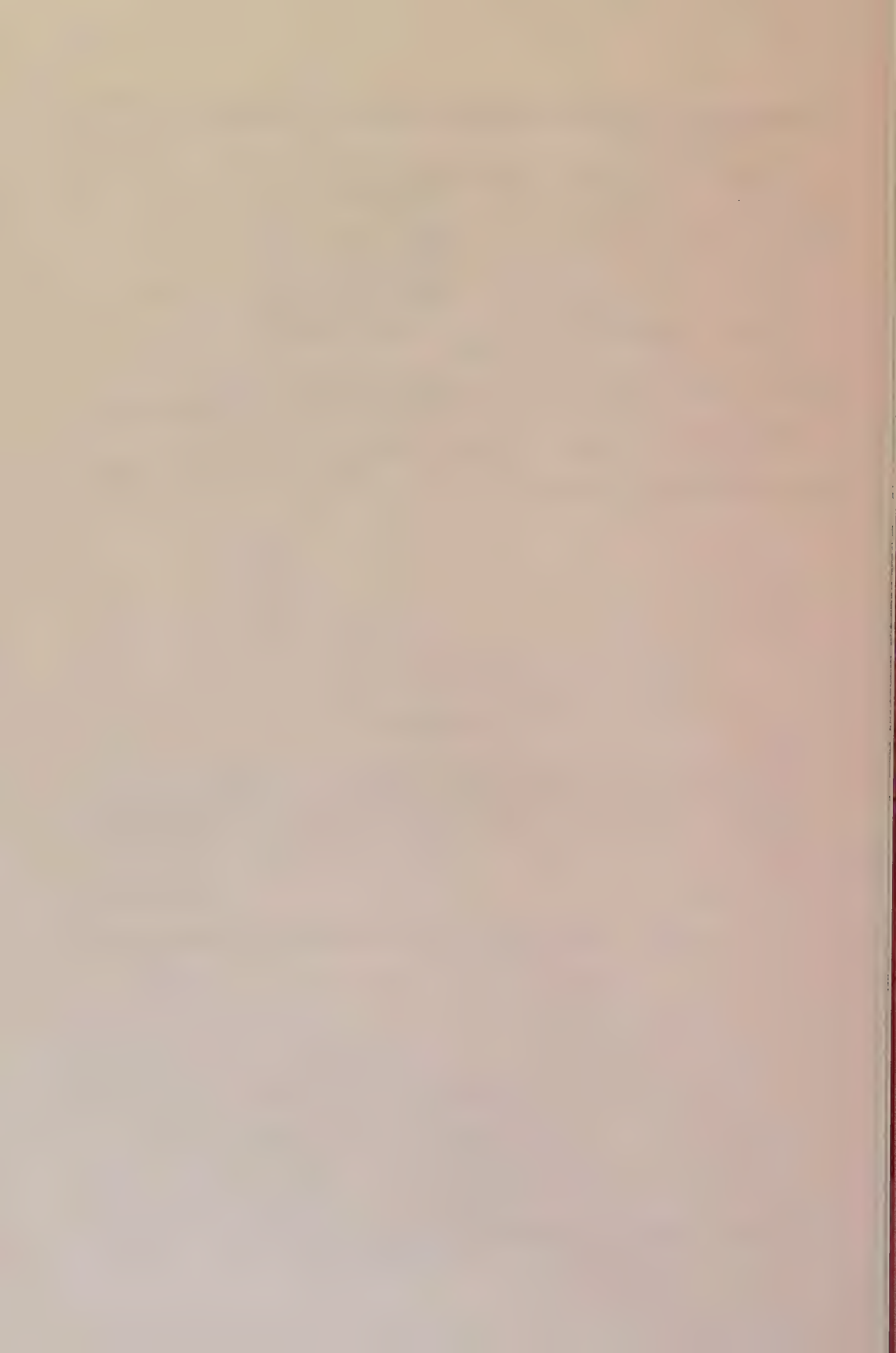
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GELATINIZATION PROPERTIES OF DIFFERENT SIZE CLASSES OF WHEAT STARCH
GRANULES MEASURED WITH DIFFERENTIAL SCANNING CALORIMETRY

by

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SUMMARY

The particle size distributions of wheat starch fractions, obtained after a sedimentation procedure, were characterized with a Coulter Counter. DSC-measurements of the starch samples showed that the small granules gelatinized within a broader temperature interval than the large granules. The gelatinization onset temperature of the small granules was lower, whereas the gelatinization peak temperature was higher compared to the large granules. The gelatinization enthalpy was independent of the particle size distribution. The enthalpy of the endothermic transition due to the amylose-lipid complex was found to be greatest for the small granules.

1. Introduction

A bimodal size distribution of starch granules has been demonstrated for wheat, barley and rye [1-5]. Baking properties, viscosity properties and enzyme susceptibility of wheat starch have been reported to depend on the granule size [1,2]. Although the starch granule size is claimed to be of such an importance, there are many conflicting opinions concerning the properties of small and large granules, which were recently reviewed by Meredith [1]. The lipid content (in mg lipids/100 g starch) has been found to be higher in the small granules [5]. Many reports claim that the amylose content is

highest in the large granules [2,6], whereas others have found the same amylose content in both small and large granules [4]. The small granules are reported to gelatinize at higher temperatures than the large ones [2,7,8].

This investigation was undertaken to reveal whether gelatinization properties as measured by differential scanning calorimetry (DSC) differ between size classes of wheat starch. The Coulter Counter was used to characterize the granule size distributions.

2. Materials and Methods

Starch was prepared from the Swedish spring wheat Amy according to the steeping method of Meredith et al. [5]. Fractionation of wheat starch into different size classes was performed according to the sedimentation procedure in water, also described by Meredith et al. [5]. The fractions which settled after 0.25 h and 16 h were used in this investigation, together with a sample of the unfractionated wheat starch. The fractions were air-dried at room temperature, and the water contents of the starch samples were measured according to the AACC-method 44-19 [9].

The particle size distribution was measured with a Coulter Counter ZB, The orifice tube used had a capillary diameter of 140 μm , which made it possible to determine particle sizes between 2.8 μm and 52 μm . In this investigation the measurements were carried out in the interval 3-36 μm . The counting solution used was Isoton II (Coulter Electronics Ltd.). Instrument calibration was made against a calibration standard (P.D.V.B. Latex) with an average particle diameter of 14.6 μm . The Coulter Counter was connected to a Channelyzer and to a microcomputer (ABC-80). The Channelyzer distributed the measurements of the particles in one hundred different channels depending upon their volume and the number of particles in each channel was obtained on the printer.

A typical primary curve representing the size distribution obtained from the Coulter Counter is given in Fig. 1A. These primary results were recalculated to describe how the starch weight of each diameter interval expressed in percent of the total starch weight varied with the particle diameter. The values in the curve in Fig. 1A were thus transformed into the curve in Fig. 1B. Each column in Fig. 1B represents the weight-% of the particles within a specific size interval (= 1 μm). The calculation of the weight-% was based

on the assumptions that the starch particles are homogeneous and that all granules have the same density.

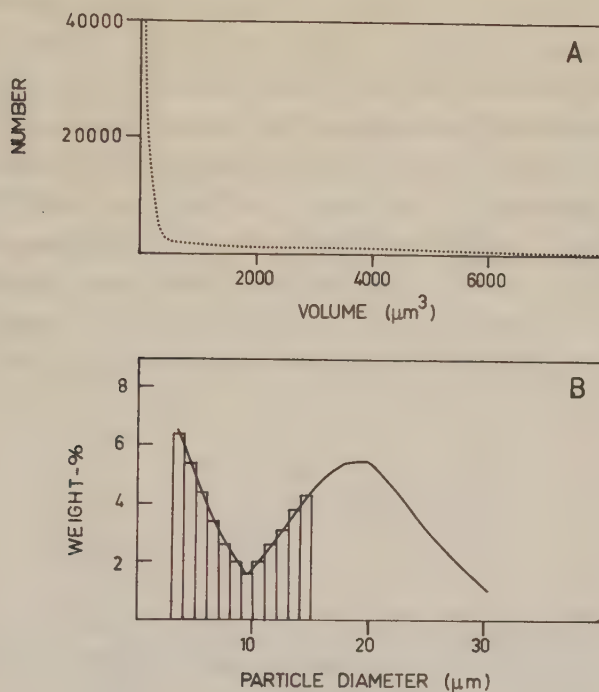


Fig. 1A. The primary curve obtained from the Coulter Counter.

Fig. 1B. The curve in Fig. 1A transformed to weight-% plotted against particle diameter.

The differential scanning calorimeter used was a Perkin Elmer DSC-2C, and the thermograms were obtained at a heating rate of $10^{\circ}\text{C}/\text{min}$. Aluminium sample pans were used with an empty sample pan as reference. The DSC-measurements were carried out for starch-to-water ratios 1:1 and 1:3, by weight.

3. Results and Discussion

When the number of starch granules was plotted against volume, the curve presented in Fig. 1A was obtained. There was thus a great number of small granules and a very rapid continuous decrease in the number of granules with increasing volumes. When, however, the particle size distribution was represented as weight-% instead of number of particles, the bimodal distribution of particle sizes was evident (Fig. 1B).

In Fig. 2 the particle size distributions of the unfractionated wheat starch sample, the fraction settled after 0.25 h sedimentation time and the fraction settled after 16 h sedimentation time are given. For the discussion the starch granules are characterized as "large" or "small" granules depending on the bimodal distribution presented in Fig. 2. For the wheat starch in question, starch granules with diameters $> 10 \mu\text{m}$ were regarded as large granules (A-granules) and starch granules with diameters $\leq 10 \mu\text{m}$ were regarded as small granules (B-granules). The weight-% of small and large granules in the investigated starch samples are given in Table I. The number of starch granules and the granule surface area per gram starch were calculated, and these results are also given in Table I. The density of the starch granules was assumed to be 1.5 and the granules were assumed to be spherical.

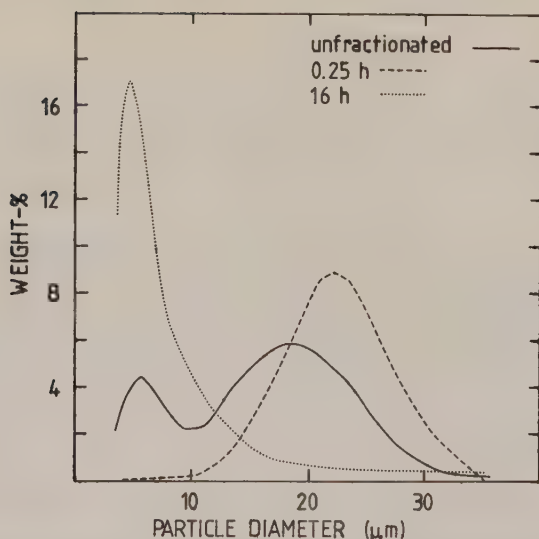


Fig. 2. The particle size distribution of the starch fractions measured by Coulter Counter.

TABLE I
Starch granule size characteristics

Wheat starch sample	Weight %	Number of granules in 1 g of starch	Granule surface area in 1 g of starch (cm ² /g)
Unfractionated			
≤ 10 μm	22.3	1.91 · 10 ⁹ (90.5%)	1541 (57.3%)
> 10 μm	77.7	0.20 · 10 ⁹ (9.5%)	1147 (42.7%)
0.25 h			
≤ 10 μm	0.8	0.36 · 10 ⁸ (19.6%)	45 (2.9%)
> 10 μm	99.2	1.48 · 10 ⁸ (80.4%)	1519 (97.1%)
16 h			
≤ 10 μm	74.2	7.95 · 10 ⁹ (98.5%)	5571 (91.8%)
> 10 μm	29.7	0.12 · 10 ⁹ (1.5%)	498 (8.2%)

The weight-% of small granules in the unfractionated starch sample (22.3%) was found to be higher than what has been reported by e.g. D'Appolonia et al. [10]. Evers [3] and Evers et al. [4] reported around 30% by weight to be granules with diameters below 10 μm. The differences in weight-% of small granules may be due to genetical differences in size distribution between wheat varieties, but environmental effects cannot be excluded. However, there is also a risk that the contribution by the small granules to the weight might be underestimated due to losses during the preparation procedure. In this investigation the starch was prepared from whole grains to reduce the amount of damaged starch granules. Losses of small granules may have occurred during the preparation, and therefore the proportion of small granules in the unfractionated starch sample, given in Fig. 2, might be somewhat underestimated. The number of granules and the granule surface area for the unfractionated wheat starch sample were in the same order as have been reported in other works [10,11]. The starch fraction, which settled after 0.25 h, contained mostly large granules (see Fig. 2), 99.2% by weight and 80.3% by number. The starch fraction, which settled after 16 h, contained mostly small granules (see Fig. 2), although the fraction was somewhat contaminated by large granules. The small granules were 74.2% by weight and 98.5% by number.

The DSC-thermograms of the starch fractions at starch-water ratios 1:1 and 1:3 are given in Figs. 3 and 4, respectively. The corresponding transition temperatures and enthalpies are given in Tables II and III. The temperatures, used to characterize the DSC-thermograms, are explained in Figs. 3 and 4.

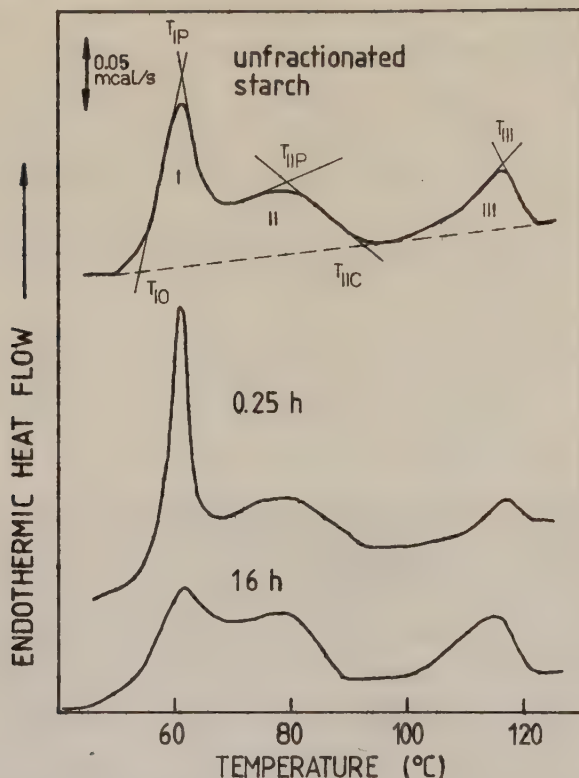


Fig. 3. DSC-thermograms of the wheat starch size classes at the starch-to-water ratio 1:1. The amount of sample is from top to below 8.45 mg, 8.66 mg and 8.21 mg.

The three endothermic transitions previously described for wheat starch at low water contents [12,13] were observed for all the starch fractions at the starch-water ratio 1:1. At the starch-water ratio 1:3 two endotherms were observed. The shape of the endotherms at the lower water content was found to be characteristic for the size distribution of the starch granules. The gelatinization endotherm (peak I) of the large granules was very narrow whereas the gelatinization endotherm of the small granules was broad (Fig. 3). The shape of the endotherm of the unfractionated starch was somewhere in between. The difference in the shape of the endotherms was found to exist also at higher water contents (Fig. 4), although the difference was not as

evident as at the lower water content.

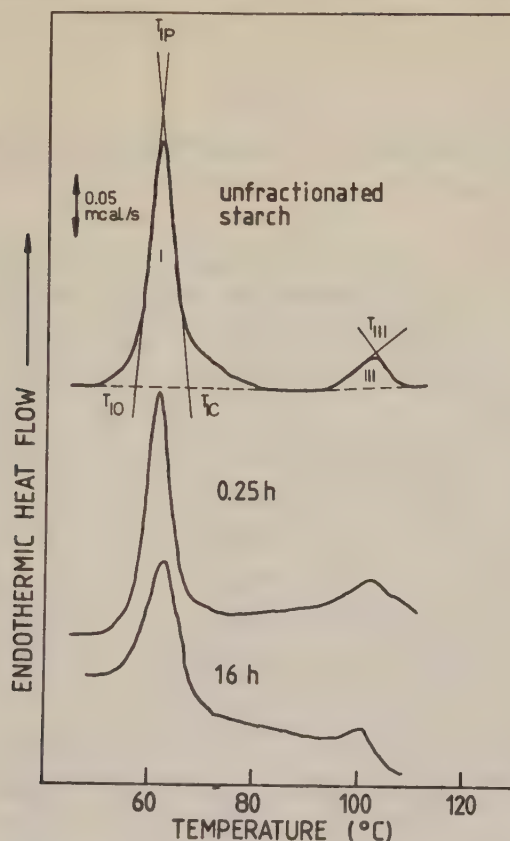


Fig. 4. DSC-thermograms of the wheat starch size classes at the starch-to-water ratio 1:3. The amount of sample is from top to below 9.86 mg, 9.43 mg and 8.38 mg.

The small wheat granules have been reported to have higher gelatinization temperatures than the large wheat granules, measured both in microscope [2] and with DSC [7]. From the measurements in this study it is evident that the small granules started to gelatinize at lower temperatures (Table II), whereas the gelatinization peak temperature was higher. Thus the gelatinization interval was broader for the small granules than for the large ones (13.7°C compared to 8.9°C at the high water content). When the water content was lowered the differences in T_{10} and T_{IP} between small and large granules still existed. T_{IIP} was almost independent of the particle size, whereas T_{IIC} was lower for the small granules. At the lower water content, the temperature interval of the two endothermic transitions was only slightly broader for the small granules. The peak temperature of the third transition,

T_{III} , was not significantly affected by the particle size at the high water content. At the low water content, T_{III} of the small granules was somewhat lower.

TABLE II
Transition temperatures of starch-fractions of different
size distributions

Starch fraction	Transition temperatures ($^{\circ}\text{C}$) ^{a,b}						T_{II}
	T_{IO}	T_{IP}	T_{IC}	T_{IIP}	T_{IIC}	ΔT^c	
Starch:water = 1:3							
Unfractionated	56.5	61.6	66.1	-	-	9.6	101.6
	± 0.4	± 0.3	± 0.4			± 0.1	± 1.0
0.25 h	56.7	61.2	65.6	-	-	8.9	102.7
	± 0.1	± 0.5	± 0.2			± 0.1	± 0.7
16 h	55.3	62.4	68.9	-	-	13.7	102.3
	± 0.2	± 0.2	± 0.5			± 0.6	± 1.6
Starch:water = 1:1							
Unfractionated	54.2	60.7	-	80.4	92.9	38.6	117.4
	± 0.3	± 0.4		± 0.6	± 0.9	± 1.2	± 0.7
0.25 h	56.6	60.4	-	79.7	93.2	36.7	116.7
	± 0.8	± 0.3		± 0.9	± 0.9	± 1.4	± 0.5
16 h	52.6	61.7	-	80.3	89.3	37.3	115.8
	± 0.7	± 0.9		± 0.5	± 1.2	± 0.5	± 0.3

^aThe nomenclature is explained in Figs. 2 and 3.

^bMean and standard deviation for triplicate determinations.

^cAt the starch-to-water ratio 1:1 ΔT is calculated as $T_{IIC} - T_{IO}$
At the starch-to-water ratio 1:3 ΔT is calculated as $T_{IC} - T_{IO}$.

The enthalpies of the transitions I and II were found to be independent of the granule size at the low starch-to-water ratio (Table III). At the water-to-starch ratio of 3:1 ΔH_I of the large granules was somewhat greater. The similarity in gelatinization enthalpies of wheat starch granules of different size classes was also reported by Stevens and Elton [7], whereas in a recent report Wong and Lelievre [14] observed greater enthalpies of small wheat

granules. Whether these conflicting results are due to differences between species and varieties are not possible to elucidate, since only very few measurements of this kind have been reported in the literature.

TABLE III
Enthalpies of the endothermic transitions of starch fractions
of different size distributions

Starch fraction	Enthalpies ^a (cal/g) starch)		
	ΔH_I	ΔH_{II}	ΔH_{III}
Starch: water = 1:3			
Unfractionated	3.03 ± 0.10	-	0.64 ± 0.07
0.25 h	3.35 ± 0.04	-	0.39 ± 0.04
16 h	3.15 ± 0.03	-	0.77 ± 0.01
Starch:water = 1:1			
Unfractionated	1.30 ± 0.03	1.48 ± 0.07	0.60 ± 0.01
0.25 h	1.35 ± 0.07	1.47 ± 0.02	0.26 ± 0.02
16 h	1.34 ± 0.11	1.46 ± 0.08	0.77 ± 0.08

^aMean and standard deviation for triplicate determinations.

The enthalpy of the third endothermic transition (Table III) varied with the granule size and was greatest for the small granules. As this transition is due to the amylose-lipid complex [12], the greater ΔH_{III} -value of the small granules could be explained by the greater lipid content found in the small wheat granules [5].

The results in this investigation clearly show that differences in gelatinization properties exist between small and large wheat granules. The gelatinization temperature of wheat starch depends on the particle size distribution, whereas the gelatinization enthalpy does not. The enthalpy of the transition due to the amylose-lipid complex varies with the granule size, which could reflect the variation of lipid content with granule size. The existence of these differences between small and large starch granules suggests that knowledge of the particle size distribution is of importance when functional properties, e.g. baking properties, of a starch are discussed. Knowledge of the starch particle size distribution should thus also be of importance in

plant breeding.

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